Levulose was added to urine and subsequently quantitatively recovered after treatment of the urine with lead acetate to remove coloring matter and inorganic salts.

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A method for obtaining suspensions of living cells from the fixed tissues, and for the plating out of individual cells.

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The method depends on the ability of living tissue cells to withstand tryptic digestion. It is applicable to such cells as will proliferate *in vitro*. Bits of tissue are cultivated in plasma, according to Burrows's modification of Harrison's technic, and when growth is well under way the preparation is flooded with trypsin dissolved in Locke's solution. Under the influence of this fluid the growing cells contract into spheres, and with the digestion of the fibrin network they are liberated. Suspensions of individual cells are thus obtained comparable to suspensions of leukocytes. When washed and plated anew in plasma the cells put forth processes and proliferate. The digestion and plating can be repeated. The method is most successful with tissues that grow loosely in strands or a network,—sarcoma, choroid, endothelium (?), connective tissue,—in distinction from those proliferating in sheets, as do the epithelial tissues. The cells of these latter are usually liberated in clumps, not as individuals.

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The carbon dioxide content of blood and alveolar air in obstructed expiration.

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In the course of a study of the effects of respiration on the circulation and more especially the modification of these effects in

asthma and emphysema, we noted (1) low pulse pressure in those cases uncomplicated by atherosclerosis, probably due to a diminished systolic volume; (2) the loud pulmonic second sound pointing towards increased resistance in the lesser circulation; (3) polycythemia, possibly a teleologic phenomenon designed to compensate for a lessened minute volume output from the left ventricle; (4) enlarged veins in the neck and cyanosis suggesting a certain amount of venous stasis. These observations led us to believe that there was considerable circulatory disturbance in conditions associated with obstructed expiration.

Due to the lack of adequate clinical material, we decided to study this subject in the experimental animal.

Dogs were used. A saturated solution of chloretone and morphine was employed for inducing anesthesia. A T-tube was inserted into the trachea. (There was no increase in the dead space.) The CO_2 in the blood was determined by the method of Barcroft and Haldane; that in the alveolar air according to Henderson's method. (Inspiratory samples were taken.) The minute volume was determined by means of a Dreser tube for collecting expired air. We first did a series of controls and found that the CO_2 content of the blood and of the alveolar air varied with the minute volume, thus corroborating the work of Haldane and Priestley. Anesthesia produced effects varying only with the ventilation. In another series we inserted a valve which worked only one way and produced obstruction to expiration for varying lengths of time. This was our method of simulating the asthmatic attack. Control determinations were made before the use of the valve was begun. We found a marked increase in the CO_2 content of both the blood and the alveolar air, especially where compensation had not occurred. In most cases there was an attempt at compensation with regard to increased ventilation, due to the sensitiveness of the respiratory center to slight rises in the CO_2 pressure in the alveolar air. As soon as the obstruction was introduced, the type of breathing changed, expiration became long drawn out, the rate slowed, the abdominal wall muscles also taking part in the attempt to force air out. We thought therefore that increased muscular work might be a factor tending to raise the CO_2 content of the blood.

We therefore did a series of controls with strychninized dogs, and got results similar to those of our controls where increased ventilation was followed by a fall in blood and alveolar air CO_2 and vice versa. To get some insight as to what was happening in the circulatory system, blood pressure observations were made simultaneously with the determination of the intrabronchial pressure.

From a study of the tracings, we can say that there is at the beginning of expiration, a preliminary squeezing out of blood from the pulmonary capillaries and veins into the left heart. This increases the systolic output from the left ventricle and the carotid pressure therefore rises for a few seconds. After a few beats, however, there is a fall in blood pressure due to the fact that the increased intrabronchial pressure exceeds the capillary pressure, and interferes with the flow through the compressed capillaries thus diminishing the return to the left auricle. Suddenly at the beginning of inspiration, when the intrabronchial pressure falls, the drop in systolic blood pressure which began toward the end of expiration is still further increased for a few beats, the depleted pulmonary capillaries taking up the blood from the right ventricle and lessening the flow to the left heart.

There is therefore a distinct interference with blood flow through the lungs, and therefore with proper aeration. Gerhardt, Minkowski, Tendloo, Stewart and Romanoff are agreed that even slight rises in intrabronchial pressure cause considerable obstruction to blood flow through the lungs, the pressure in the pulmonary capillaries and veins being little above zero.

Hoover has attempted to explain the insufficient aeration of the blood in asthmatics on a respiratory basis. He found that the CO_2 content of alveolar air rose in the asthmatic attack. He first thought that an increase in the dead space, with impaired alveolar ventilation, was the cause of this rise. But later observations showed that there was no appreciable increase in the dead space in these cases. In fact he says "the dead space is no larger in these cases than in normal persons." The cause of the disturbance in aeration could not be circulatory, for he says "to produce cyanosis by impairment in circulation, evidence of stasis must be very great."

Yet we all know that most emphysematous patients show rather well marked cyanosis without any evidence of hepatic congestion, edema, etc. He concluded from his studies "that the real difficulty of ventilation in asthma and emphysema lies in a distention of the infundibula and this fails to allow an equal diffusion of CO_2 throughout the alveolar air." Yet he adds, "when we consider that an infundibulum has a diameter of not more than 1 mm. and that in hyperdistention the infundibula cannot be enlarged more than 2 to 3 mm., it seems astonishing that when 2 liters of air distributed in such small chambers are diluted with the addition of 3.5 liters the diffusion of gases is not uniform throughout the entire mass." To which we would add that one must remember that CO_2 has a coefficient of diffusion twenty times that of oxygen.

In view of these facts, and our own studies, we are inclined to believe that the circulatory factor, contrary to Hoover, is the important one. The cough in asthmatics, with its rises in intrabronchial pressure, would certainly embarrass the lesser circulation, and the voluntary attempts to aid expiration would act in the same manner. There is here a long-drawn-out expiration with a rise in intrabronchial pressure, as a result of which resistance to the flow through the pulmonary capillaries is great. During this phase there is little opportunity for gaseous exchange. Then there follows only a short inspiration during which the CO_2 is released as the blood is taken up by the depleted pulmonary sponge. The absence of a permanent fall in blood pressure in our animal may be explained by a compensatory vasomotor contraction of the systemic arterioles, and the rise in intra-abdominal pressure. From our studies we therefore conclude that the high CO_2 content of the alveolar air in asthma, and obstructed expiration in general is due to a circulatory cause. The rise in intrabronchial pressure during the long-drawn-out expiration interferes with the free flow through the pulmonary circuit, thus causing a damming back of the blood to the venous side. There is a consequent accumulation of CO_2 in the blood with the liberation of the alveolar air CO_2 chiefly during the short inspiratory phase of asthmatic breathing.[1]

[1] We might add that a pathologic study was made of the lungs of our valve dogs by Dr. Alexander Fraser, of the Department of Pathology of University and Bellevue