shaking of the bottle is practically the same as the increased pressure recorded in the second. They will not be exactly the same, since the volumes of bottle and tubing in the two cases are not the same, but the error thus incurred is inconsequential for most purposes (cf. Boyle's law).

The calculation of the percentage saturation of hemoglobin with oxygen is made by subtracting the first reading from the second, dividing by the second reading and multiplying by 100. Suppose in the observation made at 20 mm. partial pressure of O_2 the first reading is 24 mm. and the second, 108, then

$$\frac{108 - 24}{108} \cdot 100 = 77.7\%$$ HbO and 22.3% Hb.

The result must now be plotted on coordinate paper with the percentages of HbO along the ordinates and the partial pressures of oxygen on the abscissæ.

The observation is repeated at different pressures and by joining the points, the dissociation curve for blood is obtained. Care must be taken to see that the bottle is sufficiently shaken so that the partly reduced blood absorbs all the oxygen and gives it up again with ferricyanide. It is particularly in the latter operation that care must be taken.

The influence of carbon dioxide in lowering the dissociation curve can be readily shown by the method. The procedure is as follows: After the pressure has been reduced to the desired degree in the tonometer, the latter is placed in a horizontal position so that the blood lies along the walls, free of the ends. A CO_2 generating apparatus (Kipp's) or a bottle containing this gas is then connected by suitable tubing with the free end of the tonometer, care being taken before making the connection, to fill the tubing with CO_2. To accomplish this a slow stream of the gas is maintained and the air in the tubing beyond the screw clip (*1*) is squeezed out before connecting with the CO_2 generator. The most suitable partial pressure of CO_2 to work with is 40 mm. which is secured by cautiously opening screw clip *1* until with clip *2* open, but *3* and *4* closed, the mercury descends through 40 mm. in the barometer. Clips *1* and *2* are then tightly screwed down, and the tonometer removed, the further procedure being exactly as described above.

The effect of the 40 mm. of CO_2 will be found in the above example where a partial pressure of 20 mm. O_2 was used to reduce the percentage of HbO from 77 to about 35.

THE CO_2-COMBINING POWER OF THE ALKALINE RESERVE OF THE BLOOD

After completing the estimations necessary for finding the percentage of oxyhemoglobin, in the experiments in which CO_2 is present in the tonometer, it is of interest to determine the amount of this gas with which the blood has combined. This will represent its ability to act as a buffer towards foreign acids. To perform the estimation it is necessary, however, to measure accurately the amount of blood which is removed from the tonometer to the blood gas bottle. This can readily be done by attaching a 1 c.c. pipette to the tubing of the tonometer (beyond clip *1*), a few drops of blood being allowed to escape from the pipette before delivering under the ammonia solution in the bottle, and precautions being taken not to take any of the upper layers of blood that had been exposed to full