Effect of pH on Blood Coagulation Tests, Plasma Prothrombin Time (PPT) and Partial Thromboplastin Time (PTT)

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INTRODUCTION

Blood coagulation, accomplished with many coagulation factors and through many complex reactions, is a kind of chemical reaction in vivo like the enzymatic reaction etc. and is thought to be influenced by many chemical and physical pathological conditions. With the blood conagulation test, most of the tests are described by means of the measurement of time being necessary for forming clot. They consists in chemical reactions in vitro which may be affected by many chemical and physical factors different from those taking place in vivo. In vivo, the temperature and blood pH are controlled to very narrow limits of normal range.

It has been shown previously that the blood coagulation test is markedly affected by the temperature at which the plasma specimen is incubated, and great attention has been paid to hold a constant temperature on the tests. However, the blood coagulation test is a kind of chemical reaction in vitro and it is easily thought that the coagulation test may be affected by other factors such as pH.

It was stated by Schoen[1)] that the effect of pH should not be neglected in blood coagulation test. We made several experients for the evaluation of the effect of pH on PPT and PTT and got evidence that there must be the optimal pH for the reaction of the coagulation test as well as for the stability of the coagulation factors during storage.

INSTRUMENTS

1. Stop watch
2. Water bath (37°C)
3. Astrup Ultramicro Equipment-AME 1
4. Pipete (0.1 and 0.2 ml)

5. Test tubes (10 mm in diameter, 7.5 cm in length)
6. O_2 gas cylinder
7. Humidifier with rotor meter

REAGENTS

1. Simplastin (Warner-Chilcott)
2. Cephaloplastine (DADE)
3. M/40 $CaCl_2$
4. 3.1 % sodium citrate
5. 0.01 M barbitone buffer (pH 7.6)
6. 0.2 M Tris-HCl buffer (pH 7.6)

EXPERIMENTALS

All blood specimens were obtained by means of venipuncture, using a plastic syringe from antecubital veins, to be transferred to glass tubes, which were immediately stoppered with a parafilm paper. After separation of plasma by centrifugation, the plasma was taken into other tubes to be stored air-tight with parafilm cover at their mouth. All experiments were made by the technologist without knowledge as to what the expected results were, in order to minimize bias.

Prothrombin time determinations were made by means of the 1 stage method of Quick, using a reconstituted dehydrated thromboplastin preparation.

1 : The plasma specimens which were used for the routine coagulation tests were stored in the same stoppered tubes at 4°C for 3 hours, and the pH of the plasma specimens and the height of the plasma in the tubes were measured. The relationship between the pH and the height ware shown in figure 1 which reveals that the pH of the plasma is inversely proportional to the height of the plasma. Namely, it may be suggested that because the surface area of the plasma exposed to the air allows the CO_2 in the plasma to diffuse into the air, the shorter the height of the plasma is, the more the loss of the plasma CO_2 and the rise of the plasma pH. This experiment showed that the diffussion on CO_2 from the plasma to the air makes the pH of the plasma rise and in the routine tests the plasma specimens stored for several hours have different pHs in an inverse proportion to the height of the plasma in the tubes.[2]

2 : The relationship of PPT-pH and PTT-pH are shown in Figures 2 and 3. Both values of PPT and PTT were scattered and the mean values were prolonged with the pH rise.

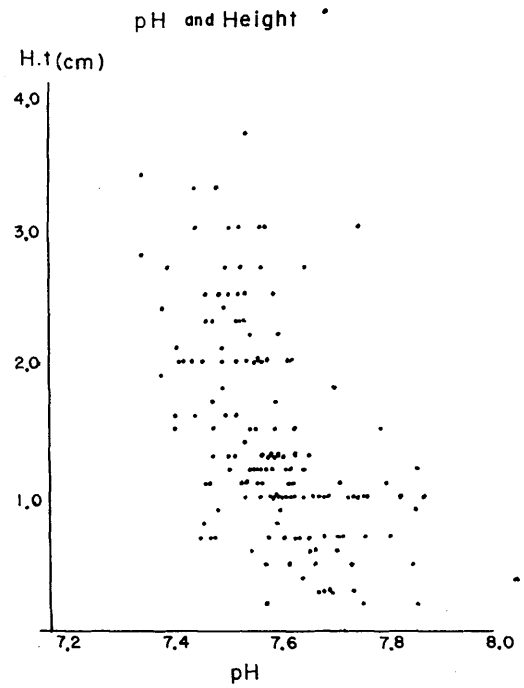

Fig. 1. Relationship between height and pH.

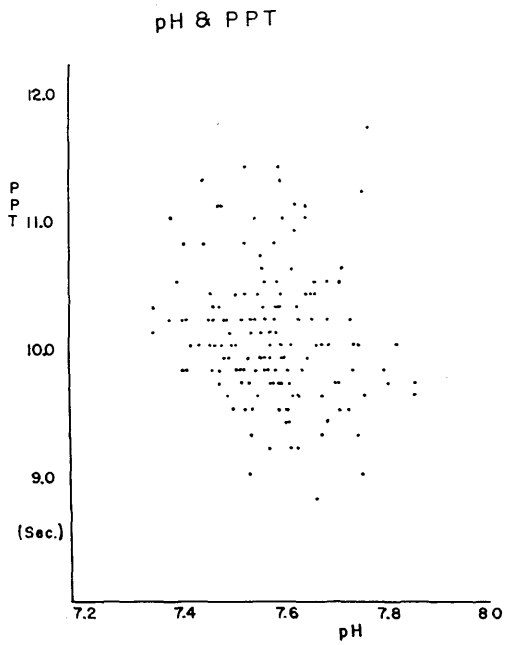

Fig. 2. Relationship between PPT and pH.

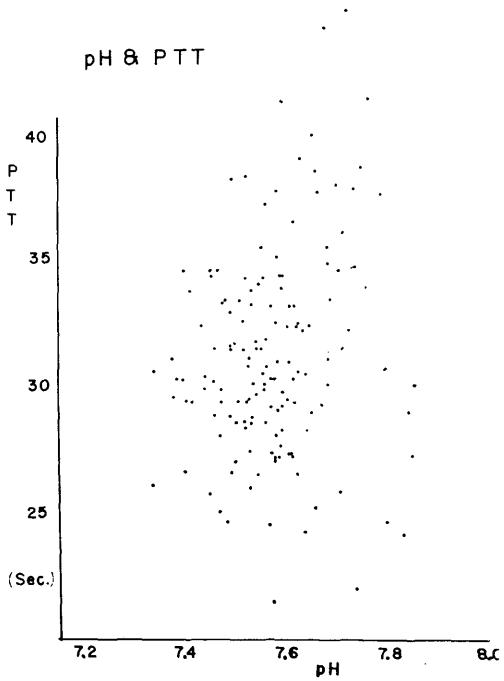

Fig. 3. Relationship between PTT and pH.

3 : We made the arrangement, illustrated in Figure 4, so that we could change the plasma CO_2 concentration. That is, O_2 gas derived from the gas cylinder was warmed through the 37°C water bath and moistened though the humidifier. The tube of CO_2 and O_2 gas mixture from the gas equilibration chamber of the Astrup microequipment AME 1 was connected to the tube of O_2 at the "A" point, shown in Figure 4. The gas mixture was warmed again and transferred into the test tubes used for the clot observation of PPT and PTT. By the valves of the Astrup gas cylinder and the O_2 gas cylinder, the CO_2 concentration of the gas mixture entering into the tube was able to be changed and the plasma pH could be changed. Twice, 0.1 ml of activated cephaloplastin and 0.1 ml of normal human plasma were added to test tube, mixed and warmed at 37°C for 5 minutes while administering the gas mixture and being vibrated. One of these plasma and cephaloplastin mixtures was measured for pH by the Astrup AME 1 type. Another was tested by the usual PTT method after adding $CaCl_2$ solution, while administrating the gas mixture into the test tubes. These procedures were performed under the different CO_2 concentrations of the gas mixture to change the plasma pH. Five samples were performed with the same procedure and we got almost the same results. One of these is shown in Figure 5. These revealed that there must be the effeet of pH and the optimal pH on the PTT like the other enzymatic reaction. Uuder the same procedure of changing plasma pH by different CO_2 concentrations, the usual PPT measurements were performed. One of these is shown in Figure 6. From this we could get no relationship between the pH and the PPT.

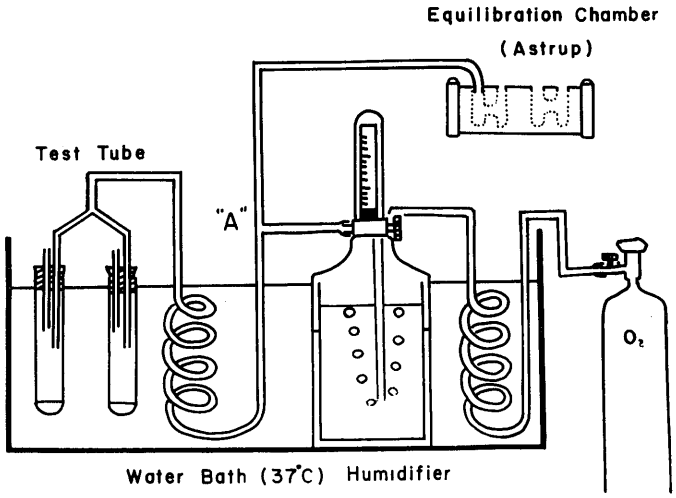

Fig. 4. Arrangement to change the plasma CO_2 concentration

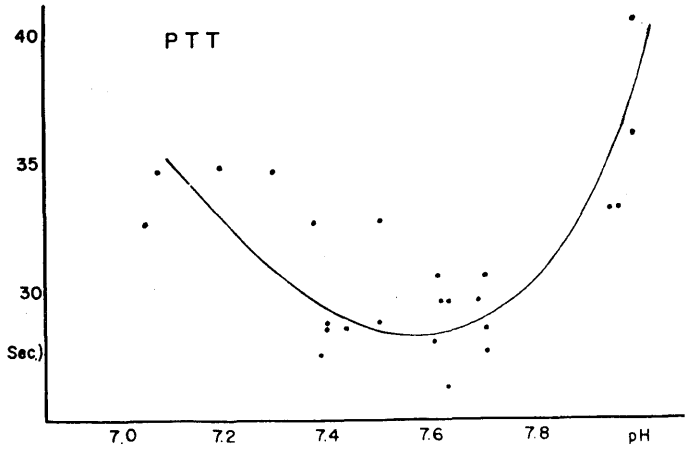

Fig. 5. Effect of pH on the PTT test (by changing the plasma CO_2 concentration)

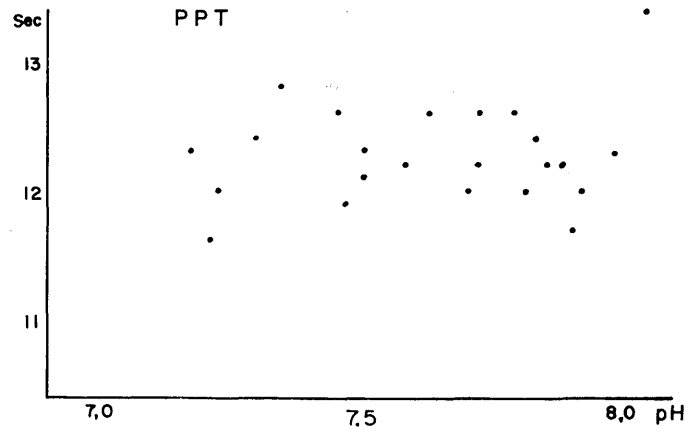

Fig. 6. Effect of pH on the PPT test (by changing the plasma CO_2 concentration)

4. The following procedures were performed in duplication. 0.3 ml of normal human plasma were transferred into seven tubes and 10 μl of 0.5N, 0.4N, 0.2N, 0.1N HCl and 10μl of 0.1M, 0.2M $NaHCO_3$ were added into each of the seven tubes. These tubes were incubated at 37°C for 5 minutes. One set was examined for pH by Astrup micro equipment AME 1. Another was tested for PTT after the addition of 37°C preincubated 0.1 ml of $CaCl_2$. The results ware shown in Figure 7. The same experiments using acid and alkali were carried out on the PPT (Figure 8). They revealed that there may be the optimal pH on the PTT and PPT.

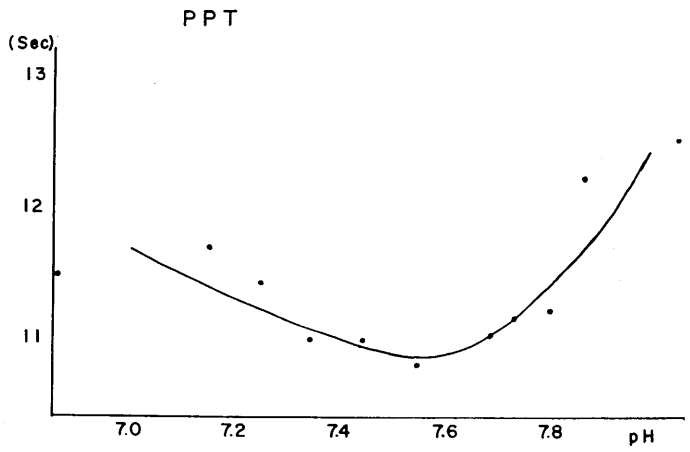

Fig. 7. Effect of pH on the PTT test (by adding acid and alkali)

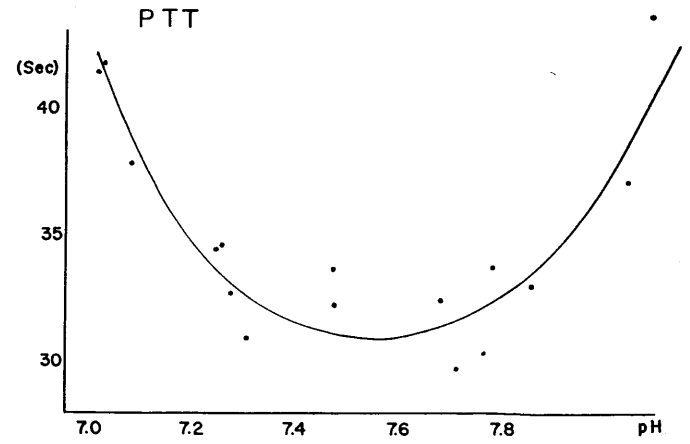

Fig. 8. Effect of pH on the PPT test (by adding acid and alkali)

5 : We tried to use buffer solution to correct the pH of the reaction system to the optimal pH (figures 9 and 11). The PPT tests using 0.01M barbitone buffer (pH 7.6) and 0.1M Tris-HCl buffer (pH 7.6) in place of distilled water to solve simplastin were done. After the PPT measurement, the solution removed from the clot was measured for pH. The results are shown in figures 9, 10, the pH could be corrected, but the values of the PPT might not be influenced comparing with the restored pH. The same experiments were done on the PTT by using 0.01 M barbitone buffer and 0.1 M tris-HCl buffer to make M/40 $CaCl_2$ solution. (figures 11, 12). Restoring the pH of the reaction system might not be able to repair the values of the PPT and the PTT.

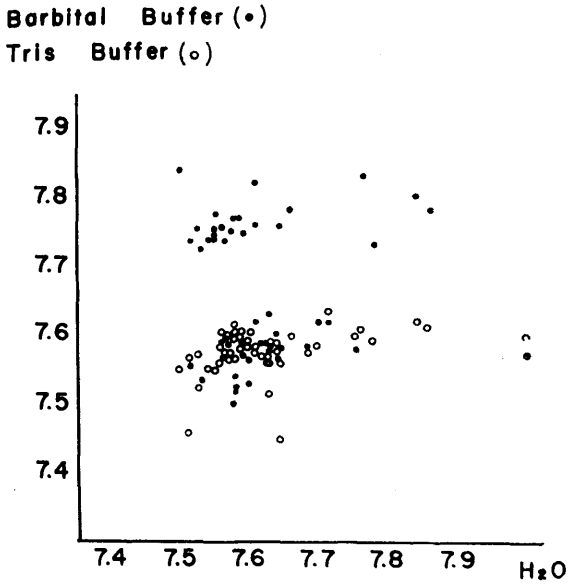

Fig. 9. Correction of pH on the PPT test

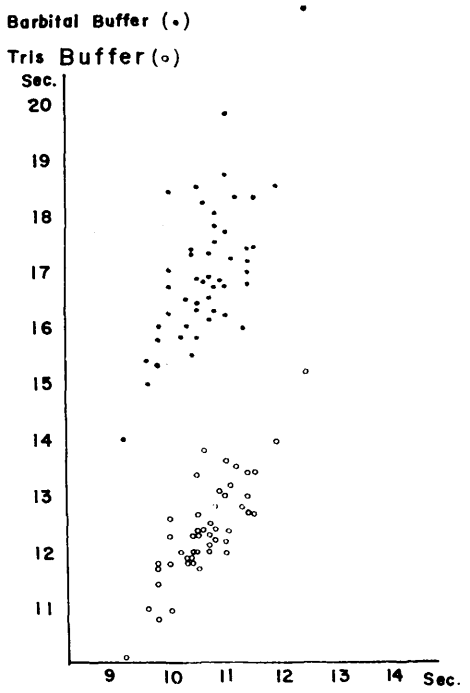

Fig. 10. Comparison between the PPT tests with buffers and with H_2O.

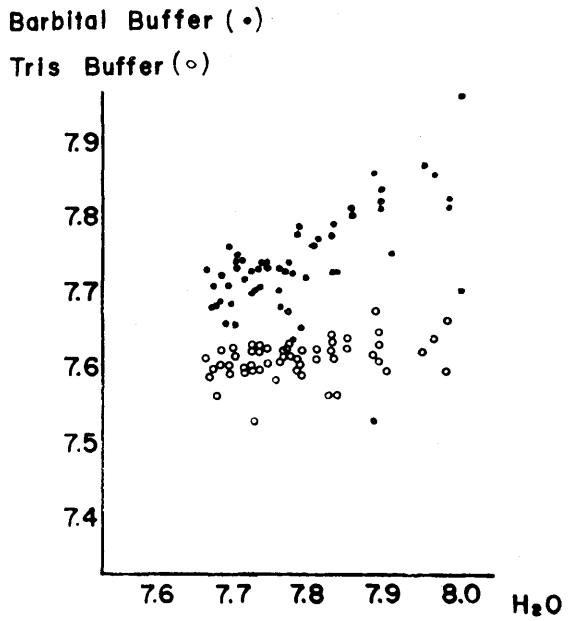

Fig. 11. Correction of pH on the PTT test

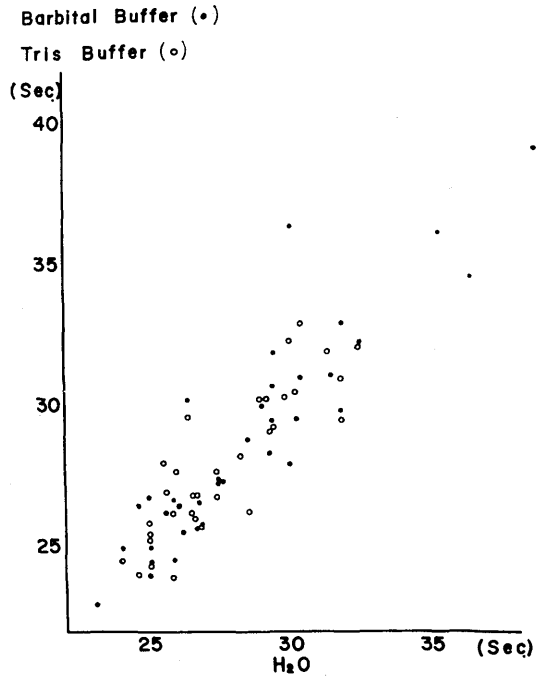

Fig. 12. Comparison between the PTT tests with buffers and with H_2O.

DISCUSSION

As shown by Balson et al.[2] experiment 1 revealed that increasing pH change seems to be related to the opportunity for CO_2 to leave the blood specimen. The plasma amounts collected for the routine studies are not equal and the PHs vary between 7.3 and 8.0. Experiments 3 and 4 showed that there must be a definite correlation of the plasma pH to the PTT and the PTT, and the variable pH of the plasma made us consider that we should be careful of the plasma pH on the routine examination. No apparent relationship between the pH and the PPT with the change of CO_2 concentration, shown in figure 6 was found. It may be caused by the relative long incubation time for CO_2 equilibrium and the short PPT time and low sensitivity of the test, or as shown by Zinsser,[3] it may be true that the change of pH by different CO_2 concentration has no effect on the PPT. However, a clear relationship between the pH and the PPT in the method using acid and alkali was found. Barrington[4] stated that the stability of Factor V in the PPT was influenced by the pH and there might be the optimal pH for storing Factor V, which was between 6.86 and 7.34. There may be the same optimal pH for the storage stability of the plasma for the PTT test because of the requirement of Factor V in the intrinsic coagulation system (PTT). As our experiments revealed, there must be optimal pH in the reaction as well as in storage. It is not clear that this in vitro phenomenon has any significance in vivo and it may be only an in vitro phenomenon without physiological value. In consideration of Crowell's data[5] showing that heparin loses activity below pH 6.7, there might be a different optimal pH for each reaction step in blood coagulation. On the other hand, we should consider the acid-base imbalance occuring in vivo as an abnormal phenomenon. Zinsser et al.[3] experimentally revealed the effect of the blood pH on the coagulation and Kaubus et al.[6] statistically showed the coagulopathy due to the acid-base imbalance. An abnormal coagulation defect due to the acid-base imbalance in the body shoud be regarded as a coagulopathy and this bleeding tendency should be able to be checked as an abnormal finding by the routine method. Although restoring pH or CO_2 could not repair the original PTT and PPT, the attempts which use the buffer to restore the plasma pH are not recommended because of the danger that the pathological plasma pH i.e. an abnormal finding, may be overlooked. In the laboratory, the technicians should take care to prevent the loss of CO_2 from the blood with a stopper and to the test as soon as possible the clinicians should be well aware that there are the effects of pH on blood coagulation and the coagulopathy due to the acid-base imbalance.

SUMMARY

It was shown that the plasma pHs were not equal in routine coagulation tests

and the values of the PPT and PTT were affected by pH. Optimal pH for the reaction as well as the storage stability of the PPT and the PTT was demonstrated by the adjustment of pH with blowing the CO_2 and O_2 gas mixture and with adding acid and alkali. Restoration of the plasma pH with buffer solution did not restore the activity of the PPT and the PTT. A discussion about the acid-base imbalance and the optimal pH was made.

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