STUDIES ON SERUM PROTEIN BINDING AND
ITS INFLUENCE ON ACTIVE BLOOD LEVEL
OF CHEMOTHERAPEUTIC AGENTS

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INTRODUCTION

It is well recognized that many chemotherapeutic agents are inactivated in vivo and may lose a part or all of their antibacterial activities. Davis has described that this phenomenon is attributed in part to the serum protein binding of the antibacterial agents. After this report, the extensive studies on protein binding of sulfonamids, penicillins, tetracyclines and other chemotherapeutic drugs have been carried out. Nevertheless these data are still incomplete in certain respects. For example, the binding rates of certain drugs are different in these reports, because the methods of assay or sera used are different. Furthermore, as Bond has pointed out, even when the same method and the same serum were used, there were individual variations in binding rate of the drug to serum protein. But there was a methodological problem in the report of Bond. So there is no report that may clarify the individual variations in the binding rate of the drug to serum protein.

On the other hand, it is also recognized that the acetylation is another factor of inactivation of certain drugs, and this process of inactivation was extensively studied on INH. Those studies indicated that the individual variations of bacteriologically active blood levels of INH were due to the differences of acetylation rates.

Consequently, the protein binding and acetylation of certain drugs may be the most important metabolic process of drug inactivation in vivo. But, in the above mentioned literatures, the relation between these two factors was never been discussed. The present author carried out the following experiments with
the intention of clarifying the facts as follow.
1. Individual difference of binding rate of the drug to serum protein.
2. Relation between acetylation rates and protein binding rates in individual subjects.
3. The effect of these two factors on the active blood level of the chemotherapeutic drugs in individual subject.

1. STUDIES ON THE FACTORS WHICH AFFECT THE SERUM PROTEIN BINDING RATE OF THE DRUG BY THE METHOD OF THE EQUILIBRIUM DIALYSIS

METHODS AND MATERIALS

The rates of binding of PAS to human albumin or serum protein were measured by equilibrium dialysis method using the apparatus shown in Fig. 1.

The internal tube is made of vinylchloride and the cellophane membrane is attached to the distal end of this tube with ARALDITE(CIBA). Two milliliter of PAS-Na solutions of various concentrations were added to 2 ml of human albumin (4 × crystallized Human Albumin, NBC.) solution of various concentrations, and these mixed solutions were incubated for 60 minutes at 37°C. The internal tube contained always 4 ml of these mixed solutions, while the
external compartment contained 8 to 60 ml of phosphate buffer solution. The
dialysis was conducted for 72 hours at 10℃, and the each apparatus was shaken
two or three times daily in order to reach an equilibrium faster.

After the dialysis the concentration of PAS in external compartment was
measured with Hitachi spectrophotometer at the wave length of 265 mμ.

The binding rate of PAS to human albumin was calculated by the following
formula.

\[
\frac{(a - b)V}{T}
\]

- a (mcg/ml): PAS concentration of external compartment, after dialysis
  in the absence of albumin solution in internal compartment
- b (mcg/ml): PAS concentration of external compartment after dialysis
  in the presence of albumin solution in internal compartment
- V (ml): Total volume of internal solution and external solution
- T (mcg): Total amount of PAS

RESULTS

1) Effect of albumin concentration

The changes of binding rate in various albumin concentration are shown
in Fig. 2. (PAS solution: 100 mcg/ml) The binding rates became higher as the

![Fig. 2. Effect of albumin concentration.](image)

albumin concentration increased. The binding rates in the cases of albumin
concentrations of 5, 10, 20, 30 and 40 mg/ml were 13.5, 22.5, 30.5, 36.0 and
37.9%, respectively.
2) Effect of drug concentration

Fig. 3 indicates the changes of binding rates by various concentrations of PAS. (Albumin solution: 40 mg/ml) In accordance with the increase of the concentration of PAS, the binding rate became lower. The binding rates in the cases of PAS concentrations of 50, 100, 200 and 400 mcg/ml were 48.0, 39.7, 30.8 and 21.8% respectively.

3) Effect of hydrogen ion concentration

When pH of external compartment was varied from 5.4 to 9.2 by using phosphate buffer solutions of different pH, the binding rates were changed as shown in Fig. 4. The binding rates in the cases of buffer solutions of pH 6.5, 7.0, 7.4, 7.7, 8.1 and 9.2 were 30.0, 33.0, 39.7, 40.8, 42.1 and 43.8% respectively. The binding rate was lowest at pH 6.5 and became slightly higher in accordance with increasing alkalinity.
4) Effect of the volume of buffer solution of external compartment

The changes of binding rate in accordance with the differences of the volumes of buffer solutions of external compartment are presented in Fig. 5. (Albumin solution: 20 mg/ml, PAS solution: 100 mcg/ml, pH: 7.4) The binding rate became lower in accordance with increasing volume of buffer solution in external compartment. The binding rates in the cases of the volume of buffer solutions of 8, 16, 24, 40 and 60 ml were 37.5, 30.5, 27.8, 24.0 and 20.8%, respectively.

2. COMPARATIVE STUDIES ON THE DIFFERENCE OF THE SERUM PROTEIN BINDING RATE OF THE DRUG IN VIVO AND IN VITRO

METHODS AND MATERIALS

To confirm whether or not the rate of protein binding of the drug in vivo is the same as measured by the method in which the drug and serum were mixed in vitro and incubated at 37°C for 60 minutes, the following studies were carried out.

The sera were obtained from ten healthy subjects (Serum 1). Then 6 g of PAS was administrated orally to these subjects and the sera were obtained two hours after the administration (Serum 2).

PAS concentrations of serum 2 were measured by the method of Bratton and Marschall\textsuperscript{17} modified by Aoyagi.\textsuperscript{18} Two milliliter of each serum 1 were diluted in 2 ml of phosphate buffer solution (pH 7.4) which contained the same concentration of PAS as that of serum 2, and then this mixture was incubated at 37°C for 60 minutes. Two milliliter of each serum 2 were diluted in the same

![Graph showing protein binding rate vs. volume of buffer solution]
volume of phosphate buffer solution of pH 7.4.

A visking tube which contained 4 ml of the above mentioned mixture of each subject was suspended in 16 ml of phosphate buffer solution of pH 7.4 as shown in Fig. 6. Dialysis was conducted for 72 hours at 10°C.

PAS concentrations of internal and external portions of each subject were measured according to the method of Bratton and Marschall modified by Aoyagi. The binding rate was calculated by the following formula:

\[
\frac{(a-b)U}{aU+bV} = \text{binding rate}
\]

where:
- \(a\) (mcg/ml): PAS concentration of internal compartment
- \(b\) (mcg/ml): PAS concentration of external compartment
- \(U\) (ml): volume of internal compartment (4ml)
- \(V\) (ml): volume of external compartment (16ml)

**RESULTS**

There were marked individual variations in the blood levels of PAS after oral administration of 6 g of PAS, as shown in Table 1. The binding rates of PAS to each serum 1 varied from 53.1% to 69.5%, the average rate being 58.7%, and these binding rates were considered to be the binding rates in vitro. On the other hand, the binding rates of PAS to each serum 2 varied from 54.9% to 70.2%, the average rate being 62.2%, and these were considered to be the binding rates in vivo. In all of the subjects, the binding rates in vivo were slightly higher than those of in vitro, but these differences were very slight.
Table 1

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Blood level of PAS (mcg/ml)</th>
<th>Binding rate in vivo (%)</th>
<th>Binding rate in vitro (%)</th>
<th>(I) — (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>120</td>
<td>56.6</td>
<td>53.6</td>
<td>3.0</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>54.9</td>
<td>53.1</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>61.8</td>
<td>58.5</td>
<td>3.3</td>
</tr>
<tr>
<td>4</td>
<td>150</td>
<td>69.9</td>
<td>65.2</td>
<td>4.7</td>
</tr>
<tr>
<td>5</td>
<td>220</td>
<td>70.2</td>
<td>69.5</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>158</td>
<td>57.7</td>
<td>54.9</td>
<td>2.8</td>
</tr>
<tr>
<td>7</td>
<td>123</td>
<td>60.6</td>
<td>58.3</td>
<td>2.3</td>
</tr>
<tr>
<td>8</td>
<td>270</td>
<td>59.8</td>
<td>55.6</td>
<td>4.2</td>
</tr>
<tr>
<td>9</td>
<td>250</td>
<td>69.9</td>
<td>62.3</td>
<td>7.6</td>
</tr>
<tr>
<td>10</td>
<td>110</td>
<td>60.6</td>
<td>56.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Average</td>
<td>177</td>
<td>62.2</td>
<td>58.7</td>
<td>3.5</td>
</tr>
</tbody>
</table>

(average 3.5%). Therefore, the measurement of the protein binding rate of PAS in vitro may be used instead of in vivo.

3. INDIVIDUAL VARIATIONS OF BINDING RATES OF PAS TO SERUM PROTEIN

METHODS AND MATERIALS

To minimize the influence of drug concentration and pH on the binding rate, the following method was used in this study.

Sera were obtained from 233 subjects with various diseases. Total protein concentration and albumin concentration of each serum were measured by refractometer and electrophoresis using cellulose acetate membrane.

Two milliliter of serum of each subject were diluted twice in phosphate buffer solution (pH 7.4) containing 200 mcg/ml of PAS. Then this mixture was incubated at 37°C for 60 minutes. The binding rate of PAS to serum of each subject was measured by the same method of equilibrium dialysis as described in experiment 2.

RESULTS

There were remarkable individual variations in the binding rates of PAS to serum protein as shown in Fig. 7. The binding rates varied from 28% to
75% (M=50.6%, S=9.3), and were distributed almost in accordance with normal distribution.

As shown in Fig. 8, there was a statistically significant correlation between albumin concentrations and binding rates, but this correlation was not so close as expected from the result of experiment 1.
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For the purpose of eliminating the influence of albumin concentration on the binding rate, the binding rates per 1 g albumin were calculated. The results of this calculation varied from 7.8 to 20.0, distributed as shown in Fig. 9. Therefore, it was clarified that there are individual variations in the binding rates of PAS to serum protein, even when the influences of albumin concentration are excepted.

4. THE EFFECT OF ACETYLATION ON THE BLOOD LEVEL OF PAS

METHODS AND MATERIALS

Seventy-one patients were selected at random for this experiment from 233 cases of which the binding rates had been previously measured. To these 71 patients 3.3 g of PAS were administrated orally, and then the sera were obtained from these subjects 2 hours after the administration. Total and free (non-acetylated) PAS concentrations of sera were measured according to the method of Bratton and Marschall modified by Aoyagi.

The acetylation rates were calculated by the following formula.

\[
\frac{T - F}{T}\quad T \text{ (mcg/ml)}: \text{Total PAS concentration} \\
F \text{ (mcg/ml)}: \text{Free (non acetylated) PAS concentration}
\]

RESULTS

The blood levels of non-acetylated PAS of each subject varied from 5 mcg/ml to 105 mcg/ml, and the distribution of these blood levels was shown in Table 2.
The acetylation rates of these subjects varied from 5% to 64.5%, and the distribution was shown in Table 3.

The relationships between these two values, non-acetylated PAS concentration and acetylation rate, were presented in Fig. 10. There was a statistically significant correlation between them (P < 0.01), that is, there is a tendency that in the cases of high acetylation rates the blood levels of PAS are significantly low. On the other hand, there was no significant correlation between the binding
rate and blood level of PAS as shown in Fig. 11.

Table 3

<table>
<thead>
<tr>
<th>Acetylation rate (%)</th>
<th>No. of Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ~ 10</td>
<td>7</td>
<td>9.9</td>
</tr>
<tr>
<td>11 ~ 20</td>
<td>15</td>
<td>21.1</td>
</tr>
<tr>
<td>21 ~ 30</td>
<td>16</td>
<td>22.5</td>
</tr>
<tr>
<td>31 ~ 40</td>
<td>16</td>
<td>22.5</td>
</tr>
<tr>
<td>41 ~ 50</td>
<td>9</td>
<td>12.7</td>
</tr>
<tr>
<td>51 ~ 60</td>
<td>5</td>
<td>7.0</td>
</tr>
<tr>
<td>61 ~</td>
<td>3</td>
<td>4.3</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>100.0</td>
</tr>
</tbody>
</table>

5. RELATION BETWEEN THE ACTIVE BLOOD LEVEL OF PAS AND THAT OF INH

METHODS AND MATERIALS

The biologically active blood levels of INH were measured six hours after oral administration of 4 mg/kg of INH in 71 individuals subjected to experiment 3 and 4 by means of the vertical diffusion method of Ogawa, using H-7 strain of atypical mycobacterium (rapid grower).

RESULTS

These patients were divided into 3 groups according to the biologically active blood levels of INH, that is, 40 cases were rapid inactivators, 22 cases intermediate inactivators and 9 cases slow inactivators (Table 4).

Table 4

<table>
<thead>
<tr>
<th>Blood level mcg/ml</th>
<th>No. of Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ~ 0.19 (Rapid inactivator)</td>
<td>40</td>
<td>56.3</td>
</tr>
<tr>
<td>0.2 ~ 0.8 (Intermediate)</td>
<td>22</td>
<td>31.0</td>
</tr>
<tr>
<td>0.18 ~ (Slow inactivator)</td>
<td>9</td>
<td>12.7</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Fig. 12. Blood levels of PAS and INH.

The correlation between the biologically active blood levels of INH and the blood levels of non-acetylated PAS measured by chemical assay was presented in Fig. 12. There was a tendency that the cases of high blood levels of INH showed also high blood levels of PAS, but this correlation was not statistically significant.

Then the PAS concentration unbound to serum protein of each subject was calculated by the following formula.

\[ F = (100 - B) \times T \]

- \( F \) (mcg/ml): Free (unbound) blood level of PAS of each subject
- \( B \) (%): The binding rate of PAS to each subjected serum
- \( T \) (mcg/ml): Total blood level of PAS

The correlation between the blood level of unbound PAS and the biologically active blood level of INH was presented in Fig. 13. There was a statistically significant correlation between these two values, \( P < 0.01 \).
6. RELATION BETWEEN THE SERUM PROTEIN BINDING RATE AND THE ACETYULATION RATE OF PAS

Relationship between the binding rate and the acetylation rate of PAS of each subject was shown in Fig. 14. There was a statistically significant correla-
tion between these two values, that is, there was a tendency that, in the cases of high binding rates, the acetylation rates were significantly low. (P< 0.01)

DISCUSSION

It is recognized that the nature of the binding of the chemotherapeutic drug to serum protein is a reversible ionic-bond, keeping the equilibrium status. Therefore, the binding rate of the drug to serum protein may vary according to the measuring methods.

The equilibrium dialysis method and the ultrafiltration method are most commonly used at present for the measurement of the rates of serum protein binding of drug. But there are certain weak points in both of these methods. For instance, in the method of equilibrium dialysis, the serum must be diluted with buffer solution, whereas in ultrafiltration method, there is a possibility of the deposition of protein molecules on the surface of the filtrating membrane and, as a result, the decrease of the permeability of drug molecules may occur. Furthermore, during the filtration, the protein concentration of the residue may increase.

On the contrary, there is no such a weak point in the ultracentrifuge method, and this method may be recognized to be the best at present. But this method of assay is unsuitable for the measurement of large number of samples.

The equilibrium dialysis method was selected for this study because of the facility of the technic. If the conditions of dialysis can be kept constant, this method of assay is much more suitable than other methods, because, as Gomi already reported, the values of binding rates measured by certain conditions of this method are nearly equal to the values measured by ultracentrifuge method.

The most influential factor on the protein binding rate measured by the equilibrium dialysis method is the volumes of the internal and the external compartments. We could prove that, if the rate of the volumes of the internal and external compartment is 1:4, the protein binding rate is nearly equal to the rate measured by the ultracentrifuge method, as a result of the studies on the binding rates of various volumes of both compartments.

The effects of protein and drug concentrations and pH to serum protein binding have been extensively studied, but the influences of the volume of the internal and external compartments and the difference between in vivo and in vitro in serum protein binding are not yet thoroughly investigated.

With regard to the effect of drug concentration on the extent of binding,
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It is known that the proportion of unbound drug increases as the drug concentration increases. In the case of certain sulfonamides, a significant decrease in the extent of binding was reported by Davis\textsuperscript{2} and Goldstein.\textsuperscript{22} Rolinson\textsuperscript{3} and Scholtan\textsuperscript{9} also reported this phenomenon in the experiment of certain penicillins. On the other hand, from the data given by Kunin\textsuperscript{5} for certain penicillins, there appeared to be little effect of drug concentration in the range 5 to 20 mcg/ml.

Rolinson reported that in the cases of benzylpenicillin and cloxacillin the percentage of unbound drug increased sharply at concentrations over 200 mcg/ml, and this phenomenon is caused by the fact that, in serum containing penicillin at such a high concentration, a significant proportion of the binding sites on the surface of protein molecules are occupied and consequently with further increases in penicillin concentration the bound proportion of the drug must fall. Then, when the same binding sites are occupied by other drugs, the bound proportion of chemotherapeutic agent may decrease.\textsuperscript{3}

This competitive effect in serum binding has been described by Kunin\textsuperscript{23} (penicillin and 250 compounds), Anton\textsuperscript{4} (sulfathiazole and phenylbutazone), and Rolinson\textsuperscript{3} (penicillins and sodiumsalicylates). But these competitive actions may occur only in the presence of high concentration of these competitive agents.

On the effect of protein concentration to the serum protein binding of the chemotherapeutic drugs, Davis\textsuperscript{2} and Rolinson\textsuperscript{3} have already reported thereon in the experiment on sulfonamides and penicillins respectively, and the same results as reported by them were obtained in this study.

The nature of serum protein binding of the drugs is considered as an ionic-bond between positively charged regions on the protein surface and negatively charged drug molecules. The changes of hydrogen ion concentration may consequently affect this ionic-bond. Davis described in the experiment of 4 different sulfonamides that in the range of pH from 6 to 8.5 all of these drugs showed a significant increase in binding with increasing alkalinity.\textsuperscript{2} This result is the same as obtained by this study in the range of pH from 6.5 to 9.1. Davis also described that at pH 12, there was no binding of sulfonamides and that the reason of these phenomena may be as follows. Proteins are "Zwitterions," that is, at the isoelectric point they have a large and equal number of positive and negative charges, and with increasing alkalinity, a net negative charge is produced by the depression of positive ionization. Hence, in strongly alkaline solution, all the drug and all the protein molecules would have only negative charges, and would repel each other, but in the intermediate range, the binding would increase with increasing alkalinity, if the importance of the increasingly ionized drug outweighed that of the decreasing
number of positive groups of protein.  

There is another factor which influences the binding rate of chemotherapeutic drugs, that is, the difference of the species of sera. Anton reported this difference using serum of human, monkey, bovine, dog, cat, rat, mouse, guinea pig and chicken. In his report, he described certain differences in binding rates among these animals, even when the same drug and the same concentration are used. Rolinson described also the same phenomenon in the experiment of benzylpenicillin and oxacillin, and emphasised that, when a number of substances are evaluated experimentally in one animal species, the assumption may not be made that the relative extent of binding of the compounds would be the same in some other species.

The individual variations of binding rates were reported only by Rolinson and Bond. Bond measured the binding rates in 10 healthy subjects after the oral administration of a certain penicillin, and proved that there are individual variations in the binding rates in each subject. But the influence of the difference of blood level of penicillin on the serum protein binding was not discussed in his paper. Since the drug concentration has a significant effect on protein binding as mentioned in experiment 1, the report of Bond seems to fail to clarify the individual variations of serum protein binding. To eliminate this influence of drug concentration, Rolinson incubated each serum of 6 healthy individuals with benzylpenicillin solution in certain concentration in vitro and measured the binding rates of each subject by the method of ultrafiltration, but the individual variations in binding rates was considered to be within the experimental error.

In the above two reports, the microbiological method of assay was used to determine the penicillin concentration in the filtrate, but the microbiological assay has some weak points in recovery and accuracy, as compared with the chemical assay. Therefore, this may be the main reason why the individual variations in binding rates could not be clarified in those two reports. Thus, the present author preferred to use PAS instead of other chemotherapeutic agents. PAS is determined very easily and the recovery is almost 100% by the above described method, and minute differences can be determined accurately in the samples.

In this report it was clarified that there are marked individual variations in the binding rates of PAS to serum protein, and the binding rates of each subject are distributed almost according to the normal distribution. The binding rate of each subject has a significant correlation with serum albumin concentration, but the binding rate corrected by the albumin concentration differs in
Each subject.

The individual difference of microbiologically active blood level of INH is considered to be due to the difference of hereditary characteristics in the metabolic process of INH, and Mitchel classified these characteristics into 3 groups, that is, slow, intermediate and rapid inactivator. Since the duration of microbiologically active blood level of INH is prolonged in the cases of slow inactivator, the therapeutic effects of INH in these cases must be superior to those in rapid inactivator, and this hypothesis was proved by many reports.

The best therapeutic regimen of tuberculosis in Japan is a combination of SM, PAS and INH, while in the U.S.A. the best regimen is that of PAS and INH. Furthermore, the incidence of peripheral neuropathy, as the side effect of INH, is more frequent in the U.S.A. than in Japan. This phenomenon is considered to be caused by the fact that the percentage of slow inactivator of INH in the U.S.A. is higher than in Japan. These differences of hereditary characteristics in the metabolic process of INH are recognised to be mainly due to the differences of acetylation rates.

Most of the drugs which have amino radicals may be inactivated in vivo by acetylation. Since PAS has amino radical as well as INH, the acetylation may take place and influence the active blood level of PAS. In such drugs the serum protein binding and acetylation may be the most important factors of inactivation, but the correlation between these two factors has never been discussed. In this report it is proved that there is a statistically significant correlation between the binding rate and the acetylation rate of PAS in each subject, that is, a subject who shows high binding rate has a tendency of low acetylation rate. Consequently, the binding rate of PAS to serum protein must also correlate the active blood level of PAS, but there is no significant correlation between the binding rate and the blood level of PAS measured by chemical assay. When this negative result is compared with the facts that the long acting sulfonamides show the high binding rate, and that the binding rates of certain tetracyclines increase in accordance with the prolongation of duration of the blood level, the binding rate of the drug may influence the duration rather than the height of blood level.

Since PAS and INH have the same amino radical, the acetylation rates of these two drugs must be equal, and the influence of the acetylation on the active blood level of these drugs must also be equal in the same individual. Oana investigated this problem in INH and Sulfathiazole but failed to prove the significant correlation between the blood levels of these two drugs, and has described that this failure may be due to the influences of serum protein binding.
of Sulfathiazole. The present author also failed to prove the correlation between the microbiologically active blood level of INH and the blood level of PAS measured by chemical assay. This failure may be due to the difference of the method of assay; that is, in the measurement of PAS the portion bound to serum protein can be measured, while in the case of INH, a part or all of the bound portion cannot be measured. When it is measured by the microbiological method, the blood level of antibiotics are greatly different according to the standard preparations. If the standards are prepared with serum the blood levels are higher than those prepared with buffer solution. The main reason of this difference is considered to be the serum protein binding.

Bond⁶ described that the use of pooled serum was unsuitable for preparation of standards for the measurement of antibiotics because of the individual variations of binding rates. So the unbound blood level of PAS in each subject is calculated and then the correlation between the microbiologically active blood level of INH and the unbound blood level of PAS is studied, and a statistically significant correlation between these two values is proved in this report. That is, one who shows high unbound blood level of PAS shows also high biologically active blood level of INH. This result may suggest that the same grade of acetylation takes place in the same individual even when different drugs are given.

The binding rates and the acetylation rates of certain drugs differ individually and are correlated to each other. These two factors of inactivation of the drug may influence the biologically active blood level of the certain chemotherapeutic agents, and in consequence play a role in their therapeutic effect. Therefore, it is a matter of great importance to measure the serum protein binding of the drug in the field of clinical medicine.

**SUMMARY**

1. The binding rate of PAS to human serum albumin measured by the method of equilibrium dialysis correlate the albumin concentration and inversely correlate the PAS concentration in the range of 50 to 400 mcg/ml.

2. The binding rate of PAS to human albumin is the lowest at pH 6.5 and increases according to the increasing alkalinity.

3. Almost the same binding rates of PAS to serum protein are obtained either in vivo and in vitro by the method of equilibrium dialysis.

4. There is an individual difference in the binding rates of PAS to serum protein even when corrected by the albumin concentration.

5. The inverse correlation is proved between the blood level and the acety-
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lation rate of PAS.

6. The inverse correlation is also proved between the binding rate and acetylation rate of PAS.

7. There is a significant correlation between the unbound blood level of PAS and the microbiologically active blood level of INH, and this result may suggest that the same grade of acetylation takes place in the same individual even when the different drugs are given.

8. The serum protein binding is one of the most important factors of inactivation of the drug in vivo as well as the acetylation, and it may influence the clinical therapeutic effect of the drugs.

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REFERENCES


