MICRODETERMINATION OF BLOOD LEVELS OF PROCaine HYDROCHLORIDE AFTER INTRAVENOUS INJECTION

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The recently aroused interest in the use of procaine hydrochloride intravenously (1-8) has demonstrated the need for an accurate determination of blood levels of the drug. The pharmacocognostics of any drug require not only a knowledge of its source, preparation, action and dosage, but also a method of determining its presence in the tissues and body fluids after its administration. Primarily, this investigation was undertaken to determine the practicability of detecting procaine in the blood stream after known amounts were injected intravenously; at the same time, some observations were made to confirm the work of others (9-11) as to the fate of procaine in the body.

A method for the determination of procaine has been described by Bandelin and Kemp (12) which is a modification of the procedure used by Bratton and Marshall (13). The method to be described is a modification of the procedure used by Bratton and Marshall for the determination of sulfanilamide in body fluids. This procedure was chosen because it is readily available for use in all laboratories and the methods described by Bratton and Marshall are already standardized. This method is applicable to procaine because the entire reaction is based upon the ability of the para-amino radicals to produce a color reaction.

In this investigation four phases of the problem of administering procaine intravenously were studied: (1) a method of determining levels of procaine hydrochloride in blood by the amount of para-aminobenzoic acid; (2) the recovery of procaine added to blood in vitro; (3) the stability of procaine in oxalated blood, and (4) the recovery of procaine from the blood of rabbits and human beings.

Method for Determining Procaine Hydrochloride in Blood

Procaine hydrochloride is para-aminobenzyl-diethylamino ethanol hydrochloride:

\[ \text{H}_2\text{N'} - \text{C} - \text{C} = \text{C} \text{H}_2 - \text{C} - \text{CO} - \text{O} - \text{CH}_2 - \text{CH}_2 - \text{N(C}_2\text{H}_5)_2 - \text{HCL} \]

Para-amino benzoic acid
Diethylamino ethanol
Break down formula

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Upon diazotization the para- amino radical is produced, forming the basis for a colorimetric test. The technic of the test follows:

a. Add 1.0 cc. of oxalated blood to be tested for procaine to 15 cc. of distilled water, allowing it to lake by standing for ten minutes.

b. Add 4.0 cc. of 15 per cent trichloracetic acid; let it stand for ten minutes, and centrifuge or filter.

c. Add 1.0 cc. of 0.1 per cent sodium nitrite solution to a 10 cc. aliquot of filtrate and wait three minutes.

d. Add 1.0 cc. of 0.5 per cent ammonium sulfamate solution to the aliquot of filtrate and wait two minutes.

e. Add 1.0 cc. of a 0.1 per cent solution of N-(1-naphthyl) ethylene diamine dihydrochloride and let stand for ten minutes.

f. The standard of procaine hydrochloride should contain 0.04 mg. per cc., and is prepared as follows: (1) by proper dilution of a 1 per cent solution of procaine, a solution of 1 cc., containing 0.04 mg., is prepared; (2) 1.0 cc. of this standard is then processed in the same manner as mentioned previously, steps "a" to "e."

g. A Bausch and Lomb, Dubosque type, colorimeter is employed.

The color is light purple, similar to that obtained in the determination of sulfonamides in the blood.

**RECOVERY OF PROCAINE ADDED TO BLOOD IN VITRO**

The second phase of this investigation was to determine whether procaine, added to oxalated human blood, would be altered in such a manner that accurate determinations could not be done. Known quantities of procaine hydrochloride were added to known quantities of blood so that the concentration was 0.02 to 0.04 mg. per cc. Table 1, which is the average of several determinations, shows that the recovery of procaine hydrochloride was satisfactory.

**TABLE 1**

<table>
<thead>
<tr>
<th>Procaine HCl Added to 1.0 cc. Blood</th>
<th>Procaine HCl Determined by This Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.020 mg.</td>
<td>0.0206 mg.</td>
</tr>
<tr>
<td>0.030 mg.</td>
<td>0.0294 mg.</td>
</tr>
<tr>
<td>0.040 mg.</td>
<td>0.0338 mg.</td>
</tr>
</tbody>
</table>

**STABILITY OF PROCAINE IN OXALATED BLOOD**

Since the recovery of procaine hydrochloride immediately following its addition to blood in vitro was satisfactory, determinations of procaine levels were made in other specimens of oxalated blood to which known amounts of procaine had been added. Determinations were made immediately, after one hour, and after twenty-four hours' storage in the ice box (4 to 6 C.) Table 2, which is the average of several determinations, shows that procaine added in vitro was stable on standing in the refrigerator, and that there was no apparent loss.
Recovery of Procaine in the Blood of Rabbits

Koster (14) has shown the presence in blood, serum and plasma of an enzyme, procaine esterase, which will hydrolyze procaine into para-amino-benzoic acid and diethyl-amino-ethanol. Since the color reaction of the test described is due to the para-amino-benzoic acid of the procaine molecule, the color produced will be proportionate to the concentration of procaine. Although Allen and Livingston (15) concluded that it was not possible to determine by any technic the amount of procaine or para-amino-benzoic acid or sulfanilamide, it is believed that in the control animals the only substance that would be found would be the para-amino-benzoic acid portion of the procaine molecule. The rabbit was chosen because, according to Koster (14), the esterase factor in the rabbit closely approximated the esterase factor of man. A 1 per cent U.S.P. solution of procaine hydrochloride, in a dose of 20 mg. per kilogram of body weight, was injected into the marginal ear vein of rabbits within five to ten seconds. The determinations of procaine levels in the blood stream were made from blood obtained from the same vein as that used for injection. This was done so as not to change the circulating amount of procaine as would occur if other areas of the body were used. Fine (16) et al. have shown that at the site of trauma there is marked capillary permeability. Further trauma to the control animals was not done so that none of the circulating procaine would permeate into the tissues.

Table 2 is an example of the results in rabbits. (Determinations in 2 human beings are added for comparison.)

**TABLE 2**

<table>
<thead>
<tr>
<th>Procaine HCL Added per cc. of Oxalated Blood</th>
<th>Procaine Recovered (Determination) Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immediately</td>
</tr>
<tr>
<td>0.020 mg.</td>
<td>0.0204 mg.</td>
</tr>
<tr>
<td>0.040 mg.</td>
<td>0.0384 mg.</td>
</tr>
<tr>
<td>0.080 mg.</td>
<td>0.078 mg.</td>
</tr>
</tbody>
</table>

Discussion

Eggleston and Hatcher (9) and Shumacker (10, 11) demonstrated that the safety of procaine hydrochloride administered intravenously is directly proportional to the decrease of the rate of the injection, the slower the injection the safer. Table 3 demonstrates the conclusion of these authors that procaine will disappear from the blood stream within twenty minutes. This rate of disappearance is so rapid that any determinations to be made will require that the blood specimen be taken either during the injection or no later than twenty minutes after the completion of the injection. The dosage of 20 mg. per kilogram of body weight was selected after trial and error; 40 mg. per kilogram of body
TABLE 3

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit 1</td>
<td>1.775</td>
<td>110</td>
<td>35.5</td>
<td>0.3227</td>
<td>0.0032</td>
</tr>
<tr>
<td>Rabbit 2</td>
<td>1.700</td>
<td>105</td>
<td>34.0</td>
<td>0.3238</td>
<td>0.0136</td>
</tr>
<tr>
<td>Rabbit 3</td>
<td>1.865</td>
<td>115</td>
<td>37.3</td>
<td>0.3243</td>
<td>0.005</td>
</tr>
<tr>
<td>Rabbit 4</td>
<td>1.600</td>
<td>99.2</td>
<td>32.0</td>
<td>0.3225</td>
<td>0.005</td>
</tr>
<tr>
<td>Rabbit 5</td>
<td>1.920</td>
<td>125</td>
<td>38.4</td>
<td>0.3228</td>
<td>0.0069</td>
</tr>
<tr>
<td>Rabbit 6</td>
<td>1.650</td>
<td>107.2</td>
<td>33.0</td>
<td>0.3247</td>
<td>0.0030</td>
</tr>
<tr>
<td>Rabbit 7</td>
<td>1.700</td>
<td>110</td>
<td>35.5</td>
<td>0.3227</td>
<td>trace</td>
</tr>
<tr>
<td>Rabbit 8</td>
<td>1.800</td>
<td>117</td>
<td>30.0</td>
<td>0.3250</td>
<td>trace</td>
</tr>
<tr>
<td>Rabbit 9</td>
<td>1.825</td>
<td>118.6</td>
<td>36.5</td>
<td>0.3249</td>
<td>0.0047</td>
</tr>
<tr>
<td>Rabbit 10</td>
<td>1.690</td>
<td>109.8</td>
<td>33.8</td>
<td>0.3246</td>
<td>trace</td>
</tr>
<tr>
<td>Male (M)</td>
<td>50</td>
<td>3600</td>
<td>200</td>
<td>0.055</td>
<td>0.0092</td>
</tr>
<tr>
<td>Male (S)</td>
<td>82</td>
<td>5800</td>
<td>500</td>
<td>0.086</td>
<td>faint</td>
</tr>
<tr>
<td>(Given in 25 minutes)</td>
<td></td>
<td></td>
<td></td>
<td>or</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

Specimens taken 30 minutes after injection contained too little procaine for determination.

weight produced instantaneous death of the animal, and dosages below 20 mg. per kilogram in rabbits gave readings that were too faint to be determined.

On the basis of the above experiment, it was thought that the amount of procaine hydrochloride to be injected into an individual should be that amount which could safely be excreted within twenty minutes. From clinical experience, utilizing a 0.1 per cent solution of procaine hydrochloride in isotonic saline solution, the following dosage was found to give the optimal benefits with the least toxic reaction. This we have called the procaine unit.

\[
\frac{4 \text{ mg.}}{\text{kilogram body weight in 20 minutes}} = 1 \text{ Procaine Unit}
\]

Utilizing the 0.1 per cent solution, then 1 cc. of solution will contain 1 mg. of procaine hydrochloride.

**Summary**

Procaine hydrochloride in blood may be determined by the method of Bratton and Marshall. The method can be used in determining blood levels during treatment.

Procaine hydrochloride in blood is stable for at least twenty-four hours when stored in the refrigerator.

Procaine hydrochloride disappears from the blood stream of rabbits and human beings within thirty minutes after intravenous injection.

The authors wish to acknowledge the cooperation and technical assistance of Dr. J. T. Myers and Mr. J. A. Ackerman, of New York, in developing this analytical method.
REFERENCES


The following are the officers for the Kansas City Society of Anesthesiology for the current year:

President: Dr. L. Lafe Bresette (reelected).
President Elect: Dr. C. R. McCubbin.
Vice President: Dr. Helen Kingsbury (reelected).
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Dr. Charles Barber, M.D., Hartford, Conn., was appointed by the President as Chairman of the Program Committee.