Cardioglobulin B Content of the Blood Plasma of Newborn Infants

By Sergio A. Bencosme, M.D., D. J. Delahaye, M.D., J. E. Fay, M.B., and N. Ozen, M.D.

SUMMARY
A comparative study was made of the cardioglobulin B content of the plasma of 26 normal adults and 20 newborn infants with the bioassay technique described by Hajdu and Leonard. The cardioglobulin B content in the plasma of infants was significantly lower than that of adults. Heparin was used as the anticoagulant in the blood samples throughout this study. It was noted that, when heparin containing phenol as a preservative was employed, the cardioglobulin B levels were lower in both groups.

Additional Indexing Words:
Cardioglobulin bioassay  Cardiac contractility

It is now 80 years since Ringer showed that the frog heart bathed in saline gradually lost its contractility and that this contractility could be restored by adding whole fresh blood or plasma. In recent years, attention has been re-directed to the cardiac active principles in plasma, largely due to the work of Hajdu and collaborators. These workers demonstrated a cardiac active principle in human plasma, and Hajdu and Leonard developed a method for its bioassay based on the staircase phenomenon in the frog heart. This principle, having a positive inotropic effect, is composed of three proteins, cardioglobulins A, B, and C, with calcium bound to the C component. It has an action similar to that of digitalis in that it can restore the tension of the hypodynamic frog heart, and in higher concentration will cause contraction. Leonard and Hajdu showed in clinical studies that the plasma concentration of cardioglobulin C was elevated in patients with severe hypertension. They also demonstrated that the concentration of this cardiac active principle is greater in patients with aortic stenosis than in those with aortic insufficiency, thereby suggesting some relationship between increased plasma concentration of cardioglobulin C and the development of increased ventricular isometric tension in systole. In a group of 17 patients with primary myocardial disease, nine had extremely low values of cardioglobulin C.

Hajdu and Leonard have defined three steps in the reaction of this plasma protein system with heart muscle cells. First, cardioglobulin B is bound to the heart muscle membrane, and then the C fraction is bound to the B fraction at the cell surface. Transport of the calcium of the cardioglobulin C complex into the cells results from the addition of a third factor, cardioglobulin A. Cardioglobulin A supplies the energy for the transfer. The cardioglobulin B content of human plasma has not been previously reported. With this background, we set up the present study using Hajdu’s method to determine the cardioglobulin B level in the plasma of a group of normal adults and newborn infants.

Cardioglobulin B Assay

Methods
The original method of Hajdu and Leonard was followed in the early stages of this work.
Later, through the courtesy of these authors, we were able to introduce into our method their most recent modifications. The reader is referred to the previously mentioned papers\textsuperscript{5, 7} for all details of technique. In this paper, only points where our method differed from that of Hajdu and Leonard will be indicated.

Early in the study, frogs were kept at 12 C as suggested by the original authors, but we found it more convenient to keep them at 4 C, and no difference in the response was observed. The frog heart cannula and the central rod were made either of glass or of jewelers' silver. The hearts were stimulated to contract with the Physiograph Stimulator, Model M.K.V.* modified to permit delivery of 15 to 25 volts for 2 msec with a frequency of 20 per minute. The voltage range of 15 to 25 was found to be satisfactory. The recording equipment was a Physiograph, Model "Four." The bioassay was performed at room temperature 26.5 ± 1.0 C. At the beginning of this work, sodium heparin containing 0.5% phenol as a preservative was used in the preparation of human plasma for assay. Because of the variability of our early results, and at the suggestion of Hajdu and Leonard (personal communication), we later used sodium heparin U.S.P. without phenol.

The amount of cardioglobulin B present in the plasma is expressed as a function of the smallest amount of material needed to induce contracture of the frog heart. In the procedure, three or more solutions containing different amounts of human plasma are assayed to determine the lowest concentration, which, on addition of excess cardioglobulin A and C, results in an end-point contracture.\textsuperscript{5} Rat plasma provides a rich source of A and C cardioglobulin and is added in amounts of 0.5 ml to the frog heart. The tracings obtained in a complete assay are shown in figure 1. A frog unit (FU) of cardioglobulin B has been defined as "the amount required to cause an end-point contracture." If this is achieved with 1.0 ml of the test plasma, then according to this definition the concentration of cardioglobulin B is 1 FU per ml, whereas if end-point contracture occurs in the presence of 0.1 ml of test plasma, the concentration of cardioglobulin B is 10 FU per ml.

**Cardioglobulin B Assay**

A total of 26 adults (14 male and 12 female) and 20 newborn infants (nine male and 11 fe-

\*Supplied by the E. and M. Instrument Company, Inc., Houston, Texas.

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male) had their plasma assayed. Heparin was the only anticoagulant used. Group 1 (heparin containing 0.5% phenol), 26 adults and 12 newborn infants were tested. Group 2 (heparin without phenol), 11 of the adults from group 1 and eight additional newborn infants were tested.

The adults ranged in age from 18 to 50 years and the infants from 12 hours to 6 days. In 24 adults, the blood pressure varied from 105/75 to 125/80. The remaining two were slightly hypertensive, with pressures of 140/105 and 140/100, respectively. Venous blood was used in all cases. In group 1, the blood was collected in a syringe previously rinsed with 1% heparin containing 0.5% phenol. In group 2, 5 ml of blood was placed in a test tube containing 500 μg of heparin, producing a concentration of 100 μg of heparin per milliliter of blood. However, because of the difficulties inherent in obtaining this volume of blood from some infants, the final concentration of heparin occasionally reached 150 μg per milliliter of blood. In the adults, the assays were usually done twice, and often several times over a period of 2 years. Because the amount of blood available from infants was limited, usually only one complete assay was possible. As an internal control, with each patient studied, we performed a simultaneous assay on the plasma of a person whose cardioglobulin B level had been previously determined. A large quantity of blood was obtained from this control subject, and an aliquot of plasma was kept frozen at −85 C. The reproducibility of the test on the control plasma was satisfactory. No change in the cardioglobulin B activity was noted in the samples that had been stored under these conditions for as long as 6 months.

**Extraction and Assay of Cardioglobulin A and C Fractions from Rat Plasma**

In preparation for later determination of the concentration of cardioglobulins A and C in human plasma, cardioglobulins A and C were extracted from rat plasma by gel filtration on Sephadex C-200, following the most recent method of Hajdu and Leonard.7 The activity and specificity of these extracts of rat cardioglobulins A and C were tested with use of a frog "cardioglobulin B heart," to which different amounts of the appropriate fraction containing cardioglobulins A and C were added, in order to obtain an end-point contracture.

<table>
<thead>
<tr>
<th>GROUP 1</th>
<th>GROUP 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEPARIN WITH PHENOL</td>
<td>HEPARIN WITHOUT PHENOL</td>
</tr>
<tr>
<td>ADULTS (26)</td>
<td>NEWBORNS (12)</td>
</tr>
</tbody>
</table>

**Figure 2**

Cardioglobulin B assay. In the two left hand columns (group 1) is shown a comparison between cardioglobulin B levels in adults and newborn infants in whom heparin with phenol was used as the anticoagulant. The newborn infants have significantly less cardioglobulin B than the adults. In the two right hand columns (group 2) is shown a comparison between cardioglobulin B levels in adults and newborn infants in whom heparin without phenol was used. As in group 1, the plasma of newborn infants has significantly less cardioglobulin B than that of adults.

*Circulation, Volume XXXVI, November 1967*
**Table 1**

*Plasma Cardioglobulin B Levels in Adults and Newborn Infants*

<table>
<thead>
<tr>
<th>Plasma Cardioglobulin B (frog units)</th>
<th>Number of observations</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Value of $t$</th>
<th>Value of $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin with phenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>26</td>
<td>17.1</td>
<td>9.0</td>
<td>4.64</td>
<td>&lt; 0.001</td>
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<tr>
<td>Infants</td>
<td>12</td>
<td>4.7</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heparin without phenol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>11</td>
<td>37.9</td>
<td>12.2</td>
<td>6.66</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Infants</td>
<td>8</td>
<td>9.2</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Results**

**Cardioglobulin B Assay**

*Group 1 (Heparin with Phenol)*

The volume of plasma needed to produce an end-point contracture of the frog heart with plasma from adults under the conditions of our assay, varied from 0.03 to 0.35 ml, whereas the volume of plasma from newborn infants required to produce an end-point contracture varied from 0.12 to 1 ml or more (fig. 2). In all instances, repeated estimations on a particular adult produced the same results. From these results it is apparent that the concentration of cardioglobulin B in adult plasma varied from 3 to 33 FU/ml, whereas that of newborn infants ranged from less than 1 to 8 FU/ml.

*Group 2 (Heparin without Phenol)*

The volume of adult plasma required to produce an end-point contracture in the frog heart varied from 0.02 to 0.04 ml, whereas the volume of plasma from newborn infants required to produce an end-point contracture ranged from 0.10 to 0.12 ml (fig. 2). Therefore, the concentration of cardioglobulin B in adults varied from 25 to 50 FU/ml, but that of newborn infants varied from 8 to 10 FU/ml.

**Assay of Cardioglobulin A and C Fraction from Rat Plasma**

The fractions obtained from the gel filtration of rat plasma showed sufficient cardioglobulin A and C activity to allow quantitative determination of these cardioglobulins. Thus, in the frog “cardioglobulin B heart” an end-point contracture was produced when 0.16 ml of the C fraction and 0.1 ml of the A fraction were used. The specificity of these fractions is evident in that no contracture was induced in the frog “cardioglobulin B heart” when either of these fractions was added without the other. Prompt contracture was induced in these hearts as soon as the required amount of the one remaining cardioglobulin was added.

**Discussion**

Hajdu and Leonard have shown that in mammalian blood there is a cardiotonic protein system composed of cardioglobulins A, B, and C, which is capable of increasing the contractility of the isolated frog heart, and in higher concentrations, inducing contracture. The results obtained in our studies are in agreement with the conclusion of those authors regarding the existence of such a cardiotonic principle.

With the advice of Hajdu and Leonard, we were able to reproduce their method, as indicated by the close agreement between the results obtained in the two laboratories, including instances in which the same blood sample was tested by both groups. However, we concur wholeheartedly with their statement that “although the method cannot be
recommended for its simplicity, it is quite dependable and accurate."

The most interesting finding in our study is the observation that the cardioglobulin B concentration in the plasma of newborn infants is significantly lower than that found in adults. This is true whether or not phenol is present in the anticoagulant solution. When heparin with phenol was employed as the anticoagulant, the mean value for cardioglobulin B in adults was 17.1 FU, whereas in newborn infants it was 4.7 FU. A similar highly significant difference was found when heparin was used without phenol. In this instance, the mean value for cardioglobulin B in adults was 37.9 FU while in newborn infants it was 9.2 FU (table 1). Despite the differences in total values observed in the two groups, the cardioglobulin B activity of the plasma of newborn infants remained about one quarter of that observed in the plasma of adults.

A comparison of the results in patients tested in groups 1 and 2 indicates that the presence of phenol in the anticoagulant somehow interferes with the bioassay, as shown by the lower values obtained in the group in which phenol was used. Phenol is present as a preservative in some commercial preparations of heparin and it is important, therefore, to obtain information from the manufacturer regarding the presence or absence of this preservative before cardioglobulin B assay.

Although the number of cases studied was small, the difference between the cardioglobulin B levels in infants and adults is of sufficient magnitude to justify further work to establish the normal values for cardioglobulin B in different age groups. Abnormalities in the content of cardioglobulins A and C have been reported in adults with cardiomyopathy and aortic valve disease. Therefore, cardioglobulin assays should be carried out in infants with heart disease.

Acknowledgment

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