Quantitative Adsorption of Antibody by the Isolated Heart and the Intensity of Cardiac Anaphylaxis

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In a recent publication from this laboratory, it was shown that the events succeeding the administration of antigen to a perfused heart taken from an ovalbumin-sensitive animal include an increase in the rate and amplitude of contraction, a decrease in coronary flow, heart block, and the release of a physiologically active material having the pharmacological and chemical properties of histamine. Separate experiments showed that the release of histamine by atrial tissues undergoing anaphylaxis could account for the observed arrhythmias and the changes in the characteristics of the atrial intracellular potentials.

In order to determine whether the observed changes were quantitatively related to the output of physiologically active material, it was necessary to establish a relationship between the antibody load of the tissue and the amount of material released. Since neither active immunization nor the passive transfer of antibodies could be expected to provide a predictable degree of sensitization, an attempt was made to determine whether the heart could be sensitized by the method of in vitro adsorption which had been successful in the case of the gut.

The present experiments show that the adsorption of 1131-labeled antiovalbumin (γ-globulin) by the heart is a definite function of the concentration of antibody used to perfuse the coronaries, and that the rate of release of histamine-like material in response to challenge with a standard dose of ovalbumin appears, within certain limits, to bear a definite relationship to the amount of antibody adsorbed by the guinea pig heart.

Methods

Antiovalbumin was produced by immunizing rabbits to four-times crystallized ovalbumin, prepared according to the method of Keckwick and Cannan. Blood was obtained by cardiac puncture, the serum separated, and the γ-globulin obtained by reprecipitating the serum four times in the presence of one-third saturated ammonium sulfate. The preparation was dialyzed against 1 per cent NaCl until sulfate-free and then dried from the frozen state. The antibody content of the soluble γ-globulin was found to be 30 per cent by means of the quantitative precipitin micromethod of Lanni et al.

A portion of the γ-globulin was labeled with 1131, according to the method of McFarlane. The specific preparation used in this study was made by treating 6 X 10⁻⁸ moles (96 mg.) of γ-globulin with 1 X 10⁻¹⁵ moles of IC1 containing 2.65 me. of 1131. Approximately 95 per cent of the radioactivity was coupled by this procedure, amounting to 1.5 I per molecule of γ-globulin. Radioactivity was detected in a well-type scintillation counter and measured with a Nuclear-Chicago Sealer. The overall efficiency of the system was 76 per cent, as determined with a Co59 source.

Hearts of normal male guinea pigs were cannulated and perfused at 37 C. with the aid of an Anderson heart perfusion apparatus, according to the methods already described. After base-line measurements of the rate, amplitude, and coronary flow had been made, the heart was perfused through the coronary circulation with a standard volume of antibody solution made up in Chenoweth’s medium. The heart was washed with antibody-free Chenoweth’s solution to remove the excess antibody and then challenged with 1 mg. of ovalbumin, instilled into the aortic cannula in a volume of 0.5 ml. Perfusates were collected before challenge as well as at various intervals during the height of the physiological reaction to the specific antigen. When the heart had recovered from the effects of the anaphylactic reaction, it was rechallenged with the same dose of antigen as had been used initially. None of the preparations used in these experiments reacted to the second dose of antigen. Finally, in order to
demonstrate that the failure of response had resulted from specific desensitization rather than from tachyphylaxis to histamine, the preparation was tested with 0.1 ml. of 10⁻³ M histamine made up in Chenoweth's solution.

All of the perfusates were stored in the deep freeze and assayed for potency upon the isolated guinea pig ileum, the contractions being referred to a calibration curve based upon the reactions of the particular test muscle to standard histamine solutions ranging in concentration, usually, between 10⁻⁸ and 10⁻⁶ M. In most cases, sufficient amounts of a given sample could be obtained so that its potency would be assayed in two dilutions. An additional test was carried out by diluting the standard solutions so as closely to bracket the unknown.

Although the release of histamine during cardiac anaphylaxis has been shown by Schild and verified by us, it may be only one of the substances released from the heart during the reaction which can produce an effect on the guinea pig ileum. The presence of significant amounts of acetylcholine and serotonin has been excluded previously, but the presence of SRS-A has not been tested for. The recent studies of Chakravarty and Broeklehurst show SRS-A to be released from the great vessels and from the heart concomitantly with histamine during anaphylactic challenge. Since the release of the two substances appears to be quantitatively correlated, the output of either, or both, would serve to gauge the intensity of cardiac anaphylaxis. For this reason, no attempt was made to separate the two individuals for assay, and the potencies are therefore expressed in terms of equivalent histamine concentration.

Results

Adsorption of Antiovalbumin

The relationship between the antibody concentration (bulk phase) employed to perfuse the coronary vessels and the amount of antibody adsorbed by the tissue was studied for bulk phase concentrations ranging from 63 to 9,400 µg./ml. and for volumes ranging from 50 to 100 ml. The effluent was collected and its radioactivity estimated. The organ was next "cleared" by being washed with an antibody-free medium, the perfusate being fractionally collected for the estimation of the rate of antibody washout. The rate of elution of radioactivity appeared to be first order; the terminal samples taken at the end of a 50-ml. washout showed a very low rate of decline of radioactivity.

At the end of the "clearing" phase, the heart was stopped by being immersed in a beaker of ice-cold Chenoweth's solution, and then placed in the well of the scintillation counter to estimate the amount of radioactive γ-globulin adsorbed. The heart was repositioned on the perfusion apparatus and, after all of the physiological variables had achieved a steady state, the organ was challenged with 1 mg. of ovalbumin and perfusates were continuously collected. At the end of the experiment, the heart was weighed and counted. The material balance, obtained by dividing the sum of the radioactivities of the perfusates and the tissue by that of the original volume of solution before perfusion, averaged 95 per cent.

The relationship of the antibody concentration in the perfusing fluid to the amount of antibody remaining per 100 mg. of wet heart is given on logarithmic coordinates in figure 1. Although figure 1 shows that the amount of antibody adsorbed is a function of
its concentration in the bulk phase, there appeared to be no parallel change in the intensities of the physiological reactions to specific challenge, suggesting that even the lowest concentration of antibody employed gave maximal release of active material. For this reason, the individual responses were pooled and are presented in table 1, (a), as mean values together with their standard errors. The results given in table 1, (a), indicate that the specific challenge increased the rate and amplitude by 36 and 56 per cent, respectively, and reduced the coronary flow by 39 per cent. The rate of output of active material was found to be equivalent to $1.2 \times 10^{-8}$ moles/Gm./min. of histamine.

Concomitantly with the physiological changes presented in table 1, (a), there appeared a transient increase in the rate of efflux of labeled material from the heart. The time-courses preceding and following challenge are presented individually by figure 2 for six hearts. In each case, the curve is designated by the bulk-phase antibody concentration used for in vitro sensitization.

In no instance was there a further detectable release of histamine-like material during rechallenge and, in all but one of the cases, there was no significant change in the rate of output of the labeled antibody as compared to the level immediately preceding rechallenge. These findings do not clarify the manner of association between antibody output and that of histamine-like material, and it is relevant in this connection to mention that the increased efflux of antibody as shown in figure 2 may appear only in response to such specific or nonspecific agents as are capable of increasing the rate or amplitude of the heart. The results presented in table 2 show the time-course of antibody output to be significantly affected by histamine, by the original challenge with antigen (curve 702), as well as by epinephrine; all of these agents produced an increase in the rate and amplitude of the cardiac response. During rechallenge with antigen, physiologically active material was not released and the output of antibody rose only insignificantly.

Experiments with Nonradioactive Antiovalbumin

Since the release of active material appeared to be relatively constant and independent of the antibody load achieved in the foregoing experiments, it was desirable to find out whether the lowest antibody load used, 1.14 \mu g./100 mg. of heart, represented the minimal amount necessary for full release under the conditions of the experiment. By reference to figure 1, it can be seen that to provide for the adsorption of 1.14 \mu g./100 mg. of heart, the bulk phase should contain about 63 \mu g. of antiovalbumin per milliliter of solution. The effects of sensitizing hearts, in vitro, with solutions containing 100 and 50 \mu g./ml. of antibody were studied according to the method previously described. The results exhibited in table 1, (b, c) clearly indicate that the release of active material from hearts perfused with 100 \mu g./ml. of antiovalbumin is not significantly dif-
different from that obtained in hearts sensitized with antibody concentrations of 60 \mu g./ml. or higher, but that perfusion with 50 \mu g./ml. effectively reduces this rate of output by about 50 per cent, within the error indicated. Although it is tempting to assume that the value equivalent to $1.0 \times 10^{-8}$ moles of histamine/Gm./min. may represent the maximal reaction of which the system is capable, it must be realized that a significant amount of the histamine is probably destroyed in its passing through the coronary circulation. The disparity between the magnitude of release and the intensity of the sequelae of anaphylaxis upon the other physiological variables is apparent from table 1. For example, the increase in heart rate appears to be the same, not only in ratio but in absolute amount, for the two antibody concentrations as well as for the histamine standard tested. In both instances, the amplitude increased during challenge with antigen, although the increase was slightly greater in the group receiving the lower concentration of antibody. In each case, the standard dose of histamine increased the magnitude of the anaphylactic response to the same absolute value as that achieved by challenge with antigen, but the percentage increase appeared to be greater because the postchallenge control amplitudes were 16 to 18 per cent lower than their corresponding preantigen controls.

The coronary flow declined during the specific challenge but the magnitude of the decline was inversely related to the antibody concentration used for sensitization. A surprising observation was that histamine increased coronary flow by 25 per cent in the group receiving the higher antibody load but that it had no significant effect on the coronary flow in the other group.

### Table 1

<table>
<thead>
<tr>
<th>Antibody in ( \mu g./ml. )</th>
<th>Test with:</th>
<th>Heart rate</th>
<th>Amplitude</th>
<th>Coronary flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Challenge</td>
<td>Control</td>
<td>Challenge</td>
</tr>
<tr>
<td></td>
<td>Heart rate</td>
<td>% Change</td>
<td>Amplitude</td>
<td>% Change</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>224.7</td>
<td>395.3</td>
<td>22.9</td>
<td>361</td>
</tr>
<tr>
<td>100 \mu g./ml.</td>
<td>O</td>
<td>± 16.8</td>
<td>± 13.5</td>
<td>± 2.9</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>± 52.9</td>
<td>± 35.9</td>
<td>± 5.5</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>± 175.1</td>
<td>± 156.2</td>
<td>± 1.64</td>
</tr>
<tr>
<td>0</td>
<td>291.4</td>
<td>427.7</td>
<td>24.6</td>
<td>36.6</td>
</tr>
<tr>
<td>50 \mu g./ml.</td>
<td>O</td>
<td>± 61.3</td>
<td>± 42.6</td>
<td>± 20.75</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>± 151.6</td>
<td>± 124.1</td>
<td>± 3.6</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>± 247.2</td>
<td>± 218.3</td>
<td>± 1.72</td>
</tr>
<tr>
<td>0</td>
<td>109.2</td>
<td>372.8</td>
<td>23.4</td>
<td>36.6</td>
</tr>
<tr>
<td>25 \mu g./ml.</td>
<td>O</td>
<td>± 66.4</td>
<td>± 44.5</td>
<td>± 18.45</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>± 105.7</td>
<td>± 87.1</td>
<td>± 1.26</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>± 372.8</td>
<td>± 336</td>
<td>± 1.56</td>
</tr>
</tbody>
</table>

Discussion

While specific passive sensitization by incubating the isolated tissues of the guinea pig with homologous\(^{10}\) and heterologous\(^{11}\) antisera, and immediate reactions to preformed antigen-antibody complexes\(^{12}\) have been reported, there was little evidence, until recently\(^{2}\), to show that antibodies actually could
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be adsorbed by tissues in accordance with simple physical-chemical principles, or that the extent of the contraction of the gut or the degranulation of mast cells was quantitatively dependent upon the antibody load achieved by in vitro adsorption.

It is evident that the basic principles of adsorption and reaction that were found applicable in the case of the gut also obtain for the heart, albeit they are obscured to some extent by the intrinsic peculiarities of cardiac physiology. The experiments described in section 1 show, quite conclusively, that the amount of total γ-globulin fixed per unit weight of tissue depends upon the concentration of this protein species in the bulk phase, although the adsorption "isotherm" appears to be of a more complex kind than that observed in the case of the gut.

The disparity between the physical and physiological isotherms has been noted before, and in the present case it may imply that the saturation of the binding sites responsible for histamine release is accomplished by antibody concentrations much lower than those needed for full saturation of the entire organ, which contains a predominance of physiologically "inert" sites. The release of histamine-like materials apparently reaches a maximum of about $1 \times 10^{-8}$ moles/Gm./min. It is noteworthy that the average value, $9.6 \times 10^{-8}$ moles/Gm./min., calculated for the 100 μg./ml. category is not greatly different from that obtained for the hearts of 18 actively immunized animals, $1 \times 10^{-8}$ moles/Gm./min., and that neither differs significantly from the average value of $1.2 \times 10^{-8}$ moles/Gm./min. obtained for hearts sensitized by bulk-phase concentrations in the range between 63 and 9,400 μg./ml. of total γ-globulin.

A rough estimate of the minimal antibody load required to release the maximal amount of histamine-like material can be made from the foregoing figures if it is recalled that the minimum must lie between the sensitizing concentrations of 50 and 100 μg. of total antibody per milliliter. The maximal release can be achieved with a bulk-phase concentration of 63.3 μg./ml., which accounts for the binding of 1.14 μg. of total γ-globulin/100 mg. of wet heart. Taking into account that only 30 per cent of the bound γ-globulin is specific antibody, the limiting load for the production of a just-maximal release is 0.343 μg. of specific antibody γ-globulin/100 mg. of wet heart.

Although the release of active material is reduced if the bulk-phase concentration is dropped from 100 to 50 μg./ml., it can be seen from table 1 that the increments in heart rate and in the amplitude of contraction appear to be effectively equal for both categories, as well as for histamine. The observation that these variables were increased by so precise a degree in all cases succeeding challenge evidently results from the fact that the tachycardia is probably atrial in origin and that the increased ventricular frequency is limited, in the present experiments, by the ability of the atrioventricular bundle to conduct impulses at a rate greater than about 36 to 40 per cent above normal. This was the maximum increment before atrioventricular block became evident. Since a relationship exists between rate and amplitude, it is not surprising that as the rate becomes limiting the extent of ventricular contraction should attain a value determined by that rate.

Wileox and Andrus reported that the effects of histamine and of antigens on the coronary flow were qualitatively parallel. It can be seen from table 1 that in the case of the in vitro sensitized heart this parallelism does not obtain.

### Table 2

<table>
<thead>
<tr>
<th>Time after treatment (min.)</th>
<th>Histamine (challenge)</th>
<th>Ovalbumin (challenge)</th>
<th>Ovalbumin</th>
<th>Epinephrine 0.05 mg.</th>
<th>Count/min./ml. perfusate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4,470</td>
<td>650</td>
<td>69</td>
<td>56</td>
<td>60</td>
</tr>
<tr>
<td>1</td>
<td>9,600</td>
<td>600</td>
<td>66</td>
<td>204</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>12,000</td>
<td>950</td>
<td>93</td>
<td>151</td>
<td>95</td>
</tr>
<tr>
<td>3</td>
<td>4,750</td>
<td>590</td>
<td>62</td>
<td>96</td>
<td>59</td>
</tr>
<tr>
<td>5</td>
<td>3,400</td>
<td>525</td>
<td>109</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Protocol from heart perfused with 702 μg. protein/ml.
Although isolated atrial tissue and comminuted ventricular muscle of actively immunized guinea pigs will release histamine when challenged with specific antigens, the principal site of histamine release by the heart may be the vascular tree itself. The evidence in support of this view is that the aorta was found by Schild\textsuperscript{7} to contain the highest fraction of releasable histamine of any tissue tested, that mast cells sensitized by the in vitro adsorption of antibodies degranulate and release histamine when specifically challenged,\textsuperscript{14} and that the mast cell population of perivascular tissues is very high.\textsuperscript{18}

### Summary

The present experiments show that the adsorption of antibodies to the guinea pig heart can be achieved by perfusing the heart with rabbit antiovalbumin solutions. The amount of radioactive antibody remaining on the tissue is a definite function of the concentration of antibody in the bulk phase instilled into the coronary circulation. When the antibody-loaded organ is challenged with ovalbumin, the efflux rate of antibody is increased; it is also increased in response to such nonspecific agents as histamine and epinephrine. Since the output rate is not affected when the heart fails to react to rechallenge with antigen, the release of antibody may be entirely dependent upon the mechanical response.

The maximal amount of histamine-like material that can be produced by the heart undergoing anaphylaxis was found to be $1 \times 10^{-8}$ moles/Gm./min., estimated as histamine; the minimal amount of antibody necessary to release this quantity was calculated to be 0.343 $\mu$g. of specific antiovalbumin/100 mg. of heart. This load can be achieved by perfusing the heart with an antibody solution containing 30 $\mu$g. of specific $\gamma$-globulin. Perfusion with an antibody solution containing 50 $\mu$g./ml. showed the amount of histamine subsequently liberated by the same dose of ovalbumin to be reduced by 50 per cent.

The rate and amplitude of contraction were increased to the same absolute values in all cases in which either ovalbumin or histamine was employed to set off the reaction, suggesting that the release of active material at even the low sensitizing doses of antibody used exceeded the ceiling of the physiological response. The decrease in coronary flow was inversely related to the dose of sensitizing antibody and bore little relation to the changes in this parameter induced by the subsequent administration of histamine.

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