Histamine Release and Intracellular Potentials During Anaphylaxis in the Isolated Heart


Although cardiovascular collapse has been recognized as a concomitant of gross anaphylaxis in many species of animal, it has been the general opinion that the circulatory irregularities associated with fatal anaphylactic shock are secondary manifestations, usually resulting from the asphyxia caused by bronchospasm. While this view, first proposed by Auer and Lewis in 1910, is perfectly plausible in the case of the guinea pig, it cannot be held with equal strength in the case of the rabbit and the dog, neither of which dies of bronchoconstriction.

The first direct proof of the involvement of the heart itself as a target organ in anaphylaxis is due to Cesaris-Demel. He showed, with excellently designed experiments, that isolated hearts of immunized rabbits and guinea pigs would react specifically to such antigens as beef serum, horse serum, cow's milk, and egg white, used previously to sensitize the intact animal. The work of Cesaris-Demel is to be distinguished from the studies of his successors in 2 important respects: first, he clearly recognized the nonspecific effects of the crude antigens he employed and therefore was very sedulous in challenging his preparations with doses below those producing nonspecific reactions; second, he rechallenged them with the same dose to show that the organ had been desensitized.

Because of the ease with which tissue anaphylaxis could be demonstrated in the guinea pig gut and uterus, it is not surprising that the technically more difficult technic of cardiac anaphylaxis should have been neglected. Most of the studies made between 1911 and 1938 are difficult to analyze immunologically because of the use of impure and sometimes bizarre—antigens which, it is realized today, could produce nonspecific reactions.

Studies upon the course of anaphylaxis in the isolated heart, made in guinea pigs, rabbits, cats, and rats, show that the severe disorganization of the heart beat, succeeding an effective dose of antigen, varies qualitatively with the species tested and quantitatively with the degree of immunization of the host. According to the comparative studies of Wilcox and Andrus, the pattern of the cardiac reaction to an effective dose of antigen, as well as to histamine, in the case of the guinea pig and the rabbit, is characterized by an increase in the rate and amplitude of contraction, a delay in atrioventricular conduction, abnormalities of origin and spread of excitation in the ventricles, and an acute reduction of the coronary flow. In the cat, on the other hand, Andrus and Wilcox found the coronary flow to be increased without a significant alteration of the heart rate. The parallelism between the effects of histamine and anaphylactic shock has been shown in many animals including the rat, in this species both the amplitude of cardiac contractions and coronary flow are abruptly reduced after an effective dose of either of these agents.

While the previous studies had demonstrated that cardiac anaphylaxis could be engendered by antigen-antibody reactions taking place in the heart itself, they had not established that any physiologically active material was elaborated during the process that could give rise to the characteristic...
changes observed.* Furthermore, in spite of the likelihood that the electrocardiographic changes were consequent upon the reaction of the coronary bed to histamine and to anaphylaxis, the independent participation of the atria was not clearly excluded.

The experiments, forming the substance of the present report, show that a physiologically active material, identified pharmacologically and chemically as histamine, is liberated during cardiac anaphylaxis and that even preparations of isolated atria will both liberate histamine and exhibit characteristic irregularities.

Methods

All experiments were made on male guinea pigs, ranging in weight between 450 and 600 Gm. The animals were sensitized by 2 intraperitoneal injections of 10 mg. of 4-times crystallized ovalbumin given on consecutive days; they were used for the perfusion experiments on the fourteenth to the nineteenth day following the second injection. The electrophysiologic experiments were performed on guinea pigs which had been injected 14 to 24 days prior to the experiment.

The balanced salt medium, used for both the electrophysiologic and the perfusion experiments, was Chowneth’s modification of Tyrode’s solution; all salts used were of analytical reagent grade, made up in water redistilled over alkaline permanganate. The solutions were gassed with 5 per cent carbon dioxide in oxygen, at 37.5 C.

Perfusion Experiments

Guinea pigs, primed with an intraperitoneal injection of 3 mg. of Na heparin in 0.9 per cent NaCl solution, were sacrificed 15 minutes later by a blow to the base of the skull. The thoracic cage was opened, the ascending aorta dissected free, and a cannula inserted and secured into the vessel. The vena cava was transected, and the heart fed in a closed weighing bottle. All experiments were made on male guinea pigs, primed with an intraperitoneal injection of 10 mg. of 4-times crystallized ovalbumin given on consecutive days; they were used for the perfusion experiments on the fourteenth to the nineteenth day following the second injection. The electrophysiologic experiments were performed on guinea pigs which had been injected 14 to 24 days prior to the experiment.

The heart was perfused for about 30 minutes from the main standpipe while several control estimates were made of the rate, amplitude, and minute perfusion volume. The organ was then perfused from the side-arm for 15 minutes and similar measurements were taken. When the 2 sets of measurements were in sufficient agreement, the heart was challenged with 0.1 ml. of a 1 per cent solution of ovalbumin in Chowneth’s solution. When the kymograph exhibited a definite change in rate or amplitude, a beaker was placed under the system to collect the effluent, the period of collection being determined by the duration of the effect. The volume of the sample and the duration were noted, and an aliquot portion was stored in the deep-freeze for subsequent bioassay. When the effect of the first challenge had worn off, the flow from the side-arm was discontinued and the perfusion from the main standpipe resumed. Twenty minutes later the side-arm flow was re-established and the organ rechallenged with ovalbumin in the manner described above. Finally, the heart was then removed, the atria trimmed away, and the organ weighed in a closed weighing bottle.

Electrophysiologic Methods

The apparatus employed to obtain simultaneous records of contractions and intracellular potentials was, in essence, a copy of that already described by Vaughan Williams.10,20 Guine pig atria were suspended horizontally in a 15 ml. bath maintained at 37.0 ± 0.1 C. Contractions were recorded with an RCA 5734 transducer and were displayed on 1 beam of a Tektronix 502 oscilloscope. Intracellular potentials were displayed on the other beam. The microelectrodes had resistances of 10 to 60 MΩ and tip potentials less than 10 nV. The cathode follower was an electrometer (ME 1400), carried on the micromanipulator. The latter permitted movements of 0.5μ to be made smoothly. The atria were driven electrically through Ag-AgCl electrodes by a stimulator which also triggered the sweep. The x-plate voltage of the oscilloscope was fed into an electronic circuit which, at the end of each sweep, enabled a pulse to be delivered to a

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*The immunologic release of histamine-like materials from resting slices of various guinea pig tissues, including the heart, has been reported by H. O. Schöld, J. Physiol. 95: 399, 1939.

†Metro Industries, Long Island City, N.Y.
Grass oscilloscope camera, modified to shift one frame in response to each pulse. Photographs were thus obtained on stationary film during consecutive heart beats, and the film was shifted automatically one frame between the beats.

Characterization

Quantitative and qualitative analyses were made by bioassay and paper chromatography, respectively.

Bioassay

The perfusates were assayed for potency, in comparison with known concentrations of histamine, upon ileal strips obtained from normal male guinea pigs. The solutions and methods used have been described in other reports from this laboratory. Further details will be found under “Results.”

Paper Chromatography

Perfusates were dried from the frozen state, redissolved in water amounting to one-tenth of the original volume, and extracted with n-butanol in a liquid extractor. The butanol was removed by vacuum distillation, the residue was taken up in water and spot-tested on filter paper with sulfanilic acid reagent. The samples were prepared by being either first concentrated in a lyophil apparatus or directly extracted from Chenoweth’s medium with butanol according to the method of McIntire, Roth, and Shaw.

Results

Characterization and Bioassay of the Active Principle Released during Cardiac Anaphylaxis

Presumptive evidence for the belief that histamine was liberated during the anaphylactic reaction was furnished by the results of preliminary pharmacologic and chemical experiments on the perfusates. Pharmacologic studies showed that the perfusates obtained during the height of the anaphylactic response to ovalbumin (fig. 1): (a) reproduced the characteristic effects when tested upon hearts of normal guinea pigs and (b) caused contraction of the guinea pig ileum but not of the rat gut (fig. 2 top); (c) their stimulating action on the guinea pig gut was blocked by pyrribenzamine (fig. 2 bottom) but not by atropine, and (d) the destruction of the active material by tissue enzymes was prevented by the presence of semicarbazide in the incubation mixture. An analysis of this evidence shows that the cardiac effects could have been produced either by histamine or serotonin, but not by acetylcholine. Serotonin and acetylcholine are excluded in (b) since both agents affect the rat gut. The presumption of histamine is strengthened in (c) by the fact that the response of the guinea pig ileum to the perfusate and to histamine, as shown in figure 2 bottom, is blocked by pretreating the muscle for 60 seconds with 0.5 µg./ml. of pyrribenzamine. The presumption of histamine is finally strengthened by the fact that the destruction of the active principle, as well as of histamine, by the monoamine oxidase system of the gut is inhibited by semicarbazide.

Identification of Histamine in Perfusates

Although the active material could not be identified by isolation and crystallization, sufficient evidence was obtained by paper chromatography to suggest that the active material was, in fact, histamine. The data displayed in table 1 make it quite evident that the amount of histamine released by a single heart at the height of its reaction to antigen would be below the limits of identifi-
Pharmacologic behavior of perfusates collected during cardiac anaphylaxis. Top. Effects of histamine and “active” perfusate (R1), on guinea pig ileum in contrast to their effects on rat gut. Neither histamine nor perfusate has a significant effect on rat muscle compared to acetylcholine (Ach). Bottom. Assay of perfusates obtained during initial challenge (R1) and rechallenge (R2) of sensitized heart exhibited in figure 1. No activity is exhibited by R2 in conformance with desensitization. Both R1 and histamine are blocked by pyribenzamine.
solutions of crystalline histamine diphosphate, also showed a single streak when tested with the sulfanilic acid reagent.

Bioassay

Owing to the limited quantities of physiologically active material yielded during the anaphylactic reaction, it was necessary to select those sections of guinea pig ileum most sensitive to histamine for use as test objects for the bioassay method. Furthermore, it was necessary to establish the form of the function that existed between the concentration of histamine and the response of the gut, in order to evaluate differences in regional sensitivity and also to provide a basis for extrapolation of outlying values, if that became necessary.

The preliminary experiments were made by removing the entire small intestine and testing 5-cm. portions for response to various doses of histamine ranging in final concentration from $2.5 \times 10^{-7}$ to $1 \times 10^{-4}$M. It was found that the sigmoidal dose-response curve to histamine could be described successfully by the logistic relationship of von Krogh,\textsuperscript{25}

$$x = k \left(\frac{y}{1-y}\right)^{1/n}$$

in which $x$ is the concentration of histamine related to a given degree of response, $k$ is a constant, $y$ is the proportion of the total contraction in the presence of $x$ molar histamine, and $1/n$ the slope of the line relating $\log x$ to $\log y/(1-y)$. Although no fundamental significance need be imputed to the relationship, i.e., adsorption, it is noteworthy that the same relationship may be derived on probabilistic grounds, as has been done by Winder et al.,\textsuperscript{28} using the probit transformation of Bliss.\textsuperscript{27}

Dose-response values obtained for the individual strips were fitted to the von Krogh equation. A preliminary inspection of the individual lines showed that they would fall into 3 natural classes according to the magnitude of their $k$ values. The $y/(1-y)$ quotients obtained at each histamine concentration were then averaged for all strips comprising a particular class, the standard errors calculated, and lines fitted visually to the array obtained for that category. The results displayed in figure 3 show that the guinea pig intestine employed in Experiment 78 could be divided into at least 3 major portions, of about 50 cm. each, according to their sensitivity to histamine. A comparison among the groups on the basis of $k$ values shows that the gradient of sensitivity of the guinea pig ileum to histamine progressively increases in the aboral direction, the median effective concentrations of histamine being $6.4 \times 10^{-7}$, $1.46 \times 10^{-6}$, and $2.7 \times 10^{-5}$M for groups A (0.50 cm. from the cecum), B (50-100 cm.), and C (100-160 cm.), respectively. The variation in sensitivity among 3 guinea pigs, assayed in the same manner as that of Experiment 78, is given in table 2.

\textsuperscript{25} Von Krogh.

\textsuperscript{26} Winder et al.

\textsuperscript{27} Bliss.

\textsuperscript{28} Winder et al.
Regional Sensitivity of Guinea Pig Ileum to Histamine

Table 2

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Group*</th>
<th>k</th>
<th>l/n</th>
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<td>$3.0 \times 10^{-2}$M</td>
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<td>B</td>
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<tr>
<td></td>
<td>C</td>
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<td>0.41</td>
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<tr>
<td></td>
<td>B</td>
<td>$1.5 \times 10^{-4}$</td>
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</tr>
<tr>
<td></td>
<td>C</td>
<td>$2.7 \times 10^{-4}$</td>
<td>0.55</td>
</tr>
<tr>
<td>79</td>
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<tr>
<td></td>
<td>C</td>
<td>$8.8 \times 10^{-4}$</td>
<td>0.24</td>
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*Distance from cecal end: A=0 to 5 cm.; B=50 to 10 cm.; C=100 to 100 cm.

Reaction of the Sensitized Heart to Perfusion with Antigen

The response of the sensitized guinea pig heart to an effective dose of antigen (ovalbumin) is characterized by 3 distinct events: an acceleration in heart rate, an increase in the amplitude of contraction, and a decrease in the coronary flow. The violence of the reaction depends upon the antibody content of the tissue and upon the challenging dose of antigen employed in the experiment. During the more intense reactions, a characteristic A-V block is observed which may wax and wane for several minutes following a single dose of antigen, as exhibited in figure 1. Usually, a second challenge with antigen is ineffective, indicating desensitization; occasionally, with smaller initial challenging doses, an effect of variable intensity may be observed when the organ is rechallenged.

The results of experiments performed on the hearts of 18 sensitized guinea pigs, given in table 1, show that the percentile changes in rate, amplitude, and coronary flow, exhibited during anaphylaxis, are quantitatively very similar to those shown after an effective dose of histamine, although it is quite ap-

parent that the absolute performance of the heart is never entirely restored to that of the initial control period after it has reacted to the antigen.

Bioassays conducted on the perfusates obtained during the height of response showed a net output of physiologically active material equivalent to $1.1 \times 10^{-8} \pm 2.1 \times 10^{-9}$ moles of histamine per gram of heart per minute. On the basis that the recovery of a known amount of histamine is only a small portion of that instilled into the preparation, it can safely be assumed that the total amount of histamine released per unit time during anaphylaxis must be several times greater than the net value found in these experiments.

Electrophysiologic Changes in Sensitized Atria Treated with Antigen or Histamine

Wilcox and Andrus11 considered that the electrocardiographic changes recorded directly from the isolated organ undergoing anaphylaxis were caused by the effects of coronary constriction on the electrical activity of the heart, and that the cardiac irregularities observed might have resulted from myocardial anoxia. Because in certain instances cardiac irregularities could be observed in the absence of a reduced coronary flow, it was suspected that these phenomena might equally well originate from the atrium. The purpose of these experiments was to determine whether sensitized atria could independently react to...
antigen and, if they could, to establish whether the acceleration in rate and the arrhythmias could be accounted for by alterations in the electrical activity of individual cells of a kind that could have been produced by locally released histamine.

**Electrical Records**

Simultaneous measurements of contractions and intracellular potentials were made in the presence of a wide range of concentrations of histamine with both rabbit atria at 31 C. and guinea pig atria at 37 C. Records were obtained from 7 pairs of atria removed from guinea pigs sensitized with ovalbumin, 14 to 24 days previously. When control records had been obtained, the atria were challenged by addition of ovalbumin directly to the organ bath. A reaction was observed in every experiment, though in 1 preparation the reaction was small; this pair of atria subsequently proved to be extremely insensitive to histamine. In all other experiments, the reaction was sufficiently strong for the atria to “break away” from the stimulus and beat spontaneously, in spite of the fact that the stimulation rate chosen was at least 50 per cent higher than the frequency of the natural pacemaker. In 2 experiments the reaction was violent enough to precipitate fibrillation. In concentrations of histamine up to $10^{-6}$M, the atria continued to follow the stimulus. There were, however, marked changes in the shape of the intracellular action potential: (a) the resting potential was reduced, (b) the overshoot was reduced, (c) the rate of rise of the action potential was greatly reduced, and (d) the tail of the repolarization phase was prolonged. There was always, also, an increase in the amplitude of the contraction which may be associated with the prolonged repolarization time. At higher concentrations of histamine, the atria began to beat spontaneously, and at still higher concentrations they fibrillated.

The effects of the antigen-antibody reaction were precisely similar, and in each experiment an attempt was made to match them with an appropriate concentration of histamine. In some experiments the atria were challenged with ovalbumin before being exposed to histamine; in others the histamine was given first, but the order made no difference in the results. In each experiment, when the antigen-antibody reaction was complete and the atria had been washed for half an hour with the control solution, the atria were rechallenged with more ovalbumin. The second challenge had no effect. At the height of the reaction to the challenging dose of ovalbumin (fig. 4C) an aliquot sample of 2 ml. was withdrawn from the bath for bioassay according to the method described in a prior section of this report. The titration of the material released by the atrium is displayed in figure 5.

Examples of records taken from 1 experiment are presented in figures 4 and 6. In figure 4A, the lower trace represents the contraction of the atria in control solution; the traces above are successive, superimposed records of the intracellular action potential at slow and fast sweep speeds. The appro-
Bioassay of material released by the shocked atria. A1 is a sample of the aliquot of material withdrawn from the bath following challenge with ovalbumin.

Figure 5

The subsequent exposure of the same pair of atria to histamine. The similarity between the records is sufficiently obvious to require no comment.

It has been suggested (see "Discussion") that the effects of an antigen-antibody reaction on the intracellular potentials of smooth muscle could have been due to the release of acetylcholine. In the atria, at any rate, as shown in figures 6E and 6F, the effects of acetylcholine are in complete contrast to those of ovalbumin and histamine, since, after acetylcholine, (a) the contractions diminish, (b) the resting potential is increased, (c) the rate of rise is not reduced but is often increased, and (d) the repolarization phase is not prolonged but shortened. Although the rate of rise in figure 6F is a little slower than that of the control at the beginning of the experiment (fig. 4B), it was no slower than the control taken after recovery from the histamine.

Discussion

The dose-response curve of a pharmacologic system generally has been considered to be the outward expression of some fundamental physical process by which a population of cells or a tissue adsorbs a given drug and reacts to it. Although it is evident, a priori, that the intensity of the reaction must depend, in some way, on the amount of active material bound, information about the character of the physical adsorption is usually lacking, and the precise relationship between the binding isotherm and the reaction curve is unknown. Recent experiments in this laboratory show that the reaction curve to antigen and histamine differs quantitatively, at least, from the experimentally determined physical adsorption isotherm describing the binding of antibody to tissue. This discrepancy arises, in part, because many cells may be capable of adsorbing more of the agent than the amount necessary to produce the maximal effect (cf. Bliss), and also because there is a distribution of sensitiveness among cells to a given dose of the stimulating agent; furthermore, antibodies may be bound to tissues which are physiologically "inert."
Most developments of the dose-response problem by toxicologic statisticians have quite properly regarded responses as being quantal because they have dealt with a clear endpoint, death. The fact that the dose-response curve of muscle and the dose-mortality curves of unicellular organisms or insects can be linearized by plotting in each case the logarithm of the probit against the logarithm of the dose does not entitle us to consider the reaction of smooth muscle cells to be quantal, as has been done by Chen et al. Since the probit and the logistic transformations fit the present data equally well, we have selected the latter transformation to express our results because it does not make any assumption about the mechanism of drug-tissue interaction.

Katsh and Marshall showed that the Schultz-Dale reaction of the sensitized guinea pig's uterus was accompanied by an increase in the frequency of the spike potentials, and by increments in the duration and amplitude of the contractions. Because similar changes were noted when these uteri were treated with acetylcholine, the authors suggested that acetylcholine might be liberated during anaphylaxis. In the absence of any supporting chemical or pharmacologic experiments, the mere similarity between the electrophysiologic reactions in the 2 situations hardly constitutes presumptive evidence for this view, particularly since other agents, such as histamine, were not ruled out.

Owing to the great difference, shown in the present study, between the effects of acetylcholine and those of anaphylaxis upon the character of the atrial action potentials, it is possible to rule out acetylcholine as a causal agent entirely on electrophysiologic grounds. The effects observed in the present study are in complete contrast with those of acetylcholine, which increases the resting potential and the overshoot and usually also increases the rate of rise of the action potential, at the same time diminishing the contractions.

Strong anaphylactic reactions and large doses of histamine precipitated fibrillation, confirming the previous observations of Rijlant and the more recent studies of Penna et al., both of whom noted a similar effect in the case of shocked rabbit atria. Bursts of action potentials would occur in which the membrane appeared to be depolarized again as soon as it had repolarized to a sufficient extent to be re-excitable. All these effects of histamine and of the anaphylactic reaction would be consistent with the hypothesis that the membrane had become excessively permeable to sodium. The diminution of resting potential, overshoot, and rate of rise could all be consequences of an accumulation of intracellular sodium.

The demonstration that histamine is released during atrial anaphylaxis by no means defines this tissue as its single source, nor does it imply that the cardiac irregularities are, in all cases, of atrial origin, but it does suggest that the sinus tachycardia, observed by Wilcox and Andrus, could have been strictly local in origin and not a sequel of coronary constriction in the ventricles.

Summary

The response of the sensitized guinea pig heart to perfusion, with an effective dose of...
ovalbumin, was found to consist of an acceleration of the rate, an increase in the amplitude of contraction, and a decrease in coronary flow, confirming the earlier observations of Wilcox and Andrus. The characteristic mechanical reaction of the isolated atria was an increase in amplitude and frequency of contraction, the more intense effects resulting in fibrillation. Electrophysiologically, atrial activity was demonstrable by bursts of action potentials, recurring as soon as the membrane had repolarized sufficiently to be re-excited. The resting potential was reduced, and the overshoot and the rate of rise of the action potential were diminished.

All of the mechanical and electrical events noted in the Langendorff heart and the atrial preparations during anaphylaxis could be reproduced precisely by an appropriate dose of histamine. Evidence for the release of histamine by both the perfused heart and the isolated atria was first obtained by pharmacologic methods and then confirmed by paper chromatography of butanolic extracts. Histamine was quantitatively estimated by a biossay method which is discussed in detail in this report.

**Acknowledgment**

We are indebted to Mrs. D. W. Peterson and Mr. Harold Moore for their assistance during certain phases of these investigations.

**Summario in Interlingua**

In confirmazione del observationes de Wilcox e Andrus, il esser constatate que le responsa del sensibilizado coerde del porco de India al perfusion con un dose efficace de ovalbumina consiste in un augmento del frequentia e del amplitude del contractione e in un reduction del fluxo coronari. Le characteristica reaction mechanic del isolato atrios essesse un augmento del amplitude e del frequentia del contractiones. Le plus intense efectos resultava in fibrillation. Electrophysiologicamente, activitate atrial essesse demonstrabile per augmentos explosive in le potentiales de action que recurrerav si tasto que le membranas essesse sufficientemente repolarisata pro que lor re-excitation essesse possibile. Le potential de reposo essesse reducita, e le resulto e le rapiditate del augmento del potential de action essesse reducita.

Omne le evencimento mechanic e electric notate in le coerde de Langendorff e in le preparato atrial durante un anaphylaxe poter esser reproducitae precisamente per un dose appropriate de histamina. Indicis pro le liberation de histamina per le coerde perfusionate si ben que per le atrios isolate essesse primo obtenite per methodos pharmacologic e confirmente subsequently per chromatographia a papiro de extractos butanolic. Le histamina essesse esstimata quantitativamente per un methodo de bioassay que es discutite in detalio in le presente reporto.

**References**

ANAPHYLAXIS IN ISOLATED HEART


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