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CARDIAC CHANGES IN GUINEA-PIGS
SENSITIZED THROUGH THE
MUCOUS MEMBRANE
OF THE DIGESTIVE TRACT

By

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The different types of anaphylactic reactions in different animals is the consequence of functional particularities of the shock-tissues and organs in which antigen-antibody reaction occurs. Which organ or tissue is involved depends on the presence of antibodies fixed there during anaphylactic sensitization. The anaphylactic reaction in guinea-pigs is manifested by bronchospasm and oedema of the bronchi (2, 3), as well as by contractions of the smooth musculature; in dogs, however, constriction of the hepatic vein during anaphylactic shock occurs, which provokes liver stasis; in rabbits, the constriction of pulmonary arterioles arrests pulmonary circulation. Therefore the clinical picture of an anaphylactic reaction can be very different, depending on the presence of antibodies in the smooth musculature, in the small blood vessels (arterioles), leucocytes, mucous glands (8, 16, 6) *i.e.*, depending on the functional particularities of the tissue in which the antigen-antibody reaction occurs or which are under the effect of pharmacodynamically active substances which are liberated during this reaction.

The anaphylactic reaction has also been examined in some isolated organs: appendix, blood vessels and auricles. *Greef, Benfey & Bokelmann* (9) found that the frequency of isolated auricles of sensitized guinea-pigs was increased if to the solu-

tion in which the auricles were emerged was added the antigen with which animals were sensitized. *Milovanović & Djuričić* (13) confirmed that the anaphylactic reaction of an isolated frog's heart had an inhibitory effect and provoked cardiac arrest.

Work by Feigen (7) as well as our results, proved that the myocardium behaved like a shock-tissue and that the isolated heart of sensitized guinea-pigs, when antigen solution was added to the perfusion solution, which provoked sensitization of the animal, reacted with an anaphylactic reaction with a characteristic picture: an increased frequency of heart beats with decrease or increase of cardiac contractions amplitudes and dissociation of the heart beats in the form of a greater or less atrioventricular arrest; separate beats of auricles from ventricles and extrasystoles of ventricular origin. The manifestations of this reaction lasted from 10 to 15 minutes, after which regular heart contraction reappeared.

Electrocardiographic changes in anaphylactic shock in animals were recorded for the first time in 1913, by *Auer & Robinson* (3). They noted atrioventricular dissociation, alterations of the ventricle complex, and various rhythm disorders. These studies were neglected for a long time, and only when an extensive study by *Mikulichich* (12) on electrocardiographic alterations in experimental anaphylactic reactions was published, did a series of other works appear. A few years later (1954) *Bickel & Fabre* (4) examined electrocardiographic changes in rabbits due to the reinjection of horse serum by use of precordial leads (V_1 and V_5). The authors noted ischaemia, infarction of the posterior wall of the heart, disorders of rhythm, atrioventricular dissociation, blockage and a picture which resembled that of the WPW syndrome during anaphylactic shock, provoked in rabbits with large doses of horse serum. *Arnovljević & Spužić* (1) noted electrocardiographic changes in clinically unobservable anaphylactic reactions in rabbits and guinea-pigs. These changes were transitory and reversible, and were usually visible in final area of the ventricular complex (denivelization of the ST segment

and lowering of the T waves up to negativity). *Halpern* & his co-workers (10) considered that electrocardiographic changes during anaphylactic shock may be attributed to coronary changes.

Starting from the fact that the myocardium of guinea-pigs behaved like shock-tissue, we decided to find out if sessile antibodies could be fixed to the heart muscle, where sensitization of animals took place through the digestive tract and if—by provoking an anaphylactic reaction in these experimental conditions—electrocardiographic changes might be developed.

METHODS

Experiments were carried out in guinea-pigs of both sexes, of 350–600 g in weight. Crystallized ovalbumin (Merck) was used for sensitization. It was introduced into the stomach by a probe, 10 mg/100 g body weight daily for 10 days.

Further investigations were carried out in sensitized animals by two methods: One group of guinea-pigs (A) was subjected to anaphylactic reactions of the isolated heart by Langendorff's method and by Schultz-Dale test of the small intestine. The second group of guinea-pigs (B) was subjected to clinical latent and manifest anaphylactic reactions. Electrocardiographic recordings of the heart beats were carried out with needle-shaped subcutaneous electrodes in three standards leads (D₁, D₂, D₃). 24–48 hours later all surviving animals of this group were subjected to the same experiments as the guinea-pigs of the B group. Each group had also a corresponding control group, *i.e.*, a group of non-sensitized animals which were subjected to the same treatment.

RESULTS

Experimental group A. Between 5 and 10 days from the last introduction of ovalbumin into the stomach we put the guinea-pigs to death (12) without any anaesthesia, isolated their heart and prepared it according to the Langendorff's method. The modified Tirod's solution (Chenoweth-Koelle) was used as perfusion solution. Ten minutes after the re-establishment of regular heart beats, we added to the perfusion solution crystal ovalbumin—antigen which was used for the sensitization of animals through their digestive tract. In a certain number of guinea-pigs (5) a positive anaphylactic reaction of the

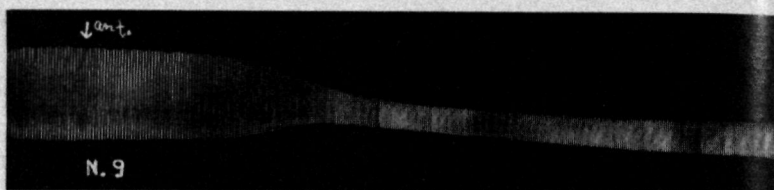


Fig. 1.

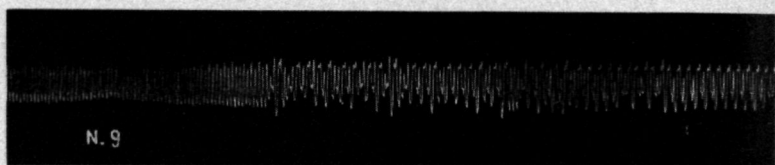


Fig. 2.

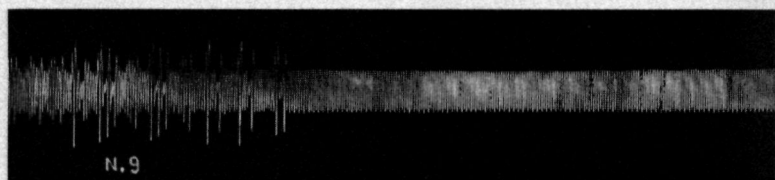


Fig. 3.

isolated heart was obtained, with the following changes of the heart beats:

1. Increased frequency of heart beats and decreased cardiac contraction amplitudes in the moment of the contact of antigen with the heart (Fig. 1).

2. Dissociation of heart beats two or three minutes later; separated beats of auricles and ventricles, atrioventricular detention and extrasystoles of ventricular origin (Fig. 2).

3. Finally, after these alterations, which lasted for 5-6 minutes the heart beats became regular, as before the administration of antigen (Fig. 3).

Parallel to the examination of the anaphylactic reaction of the isolated heart we also carried out the Schultz-Dale test with the small intestine. In all guinea-pigs which showed a

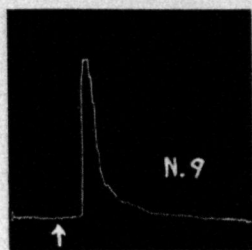


Fig. 4.

positive anaphylactic reaction of the isolated heart the Schultz-Dale test was also positive (Fig. 4).

In animals in whom an anaphylactic reaction of the isolated heart after the addition of antigen, did not occur, the Schultz-Dale test was negative.

In a certain number of guinea-pigs the anaphylactic reaction of the isolated heart showed an increased frequency of heart pulsation and decrease of contraction amplitude, after which normal heart beats reappeared. No dissociation of the heart was noted.

The control group of guinea-pigs, *i.e.*, non-sensitized guinea-pigs, showed no alterations after the addition of ovalbumin to the perfusion solution.

Our investigations were undertaken with the object of confirming whether or not there was a possibility of fixing sessile antibodies to the myocardium during sensitization through the digestive tract. After proving the existence of this possibility, which can be seen from our results, we began to study electrocardiographic changes in animals which were sensitized in the same way as the guinea-pigs in group A, *i.e.*, through the digestive tract (group B).

Experimental group B. All the guinea-pigs in this group were sensitized by a gastric probe for 10 days by the daily introduction of ovalbumin (10 mg/100 g body weight). From seven to ten days later we introduced antigen into the stomach of a certain number of guinea-pigs (B_1) with a gastric probe

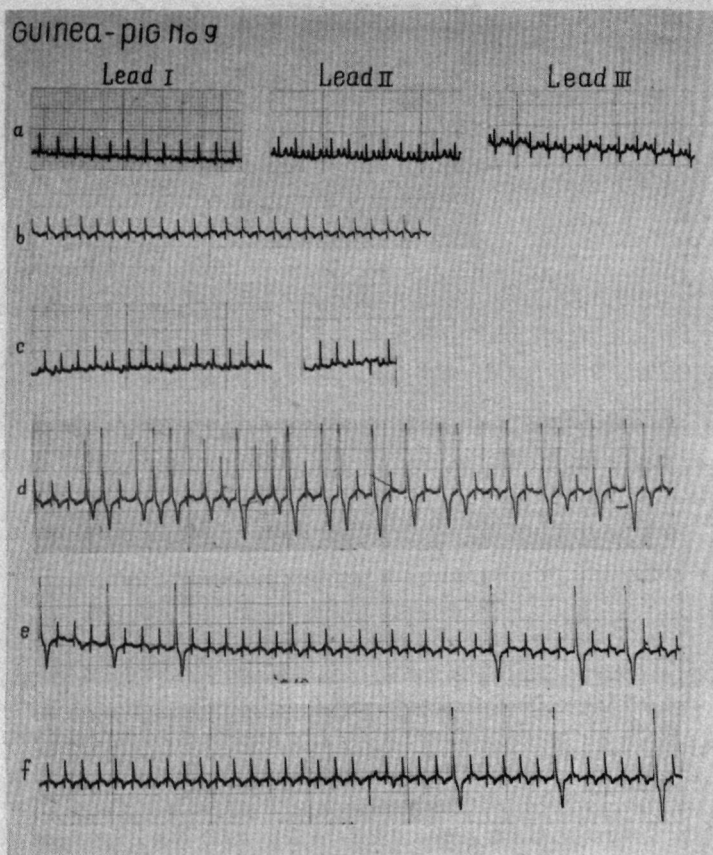


Fig. 5.

(10 mg of ovalbumin/100 g body weight). The other guinea-pigs (B_2) received intravenously only 1 ml of 0.1 per cent ovalbumin solution. The animals were not narcotized.

The guinea-pigs in B_1 group did not manifest visible signs of anaphylactic reaction after the introduction of ovalbumin with a gastric probe. All the animals survived. 24-48 hours later we put them to death and examined the anaphylactic reaction of the isolated heart and intestine.

Electrocardiographic recordings were made before and

after the introduction of antigen, and every three minutes in the following 3.5 to 4 hours.

Of 18 guinea-pigs (B_1) in which antigen was introduced with a gastric probe after sensitization, 7 manifested electrocardiographic changes: in 4 guinea-pigs these changes appeared 30–40 minutes after the introduction of antigen, and in 3 cases the same type of changes was noted 100–110 minutes later.

In 4 guinea-pigs electrocardiographic changes were manifested by ischaemia, altered cardiac frequency and rhythm, as well as early ectopic ventricular contractions (Fig. 5).

Fig. 5 shows the analysis of the electrocardiographic changes recorded in guinea-pig No. 9.

- a. Control before administration of antigen: Sinus rhythm; frequency 333/min.
- b. 30 min.: Signs of ischaemia (D_1); sinus rhythm; frequency 357/min.
- c. 88 min.: Signs of ischaemia also in D_{II} , with early ventricular contractions; frequency 349/min.
- d. 106 min.: Salvo of early ectopic contractions of the right ventricle (D_I); frequency of ventricular contractions 344/min.
- e. 107 min.: Separate early ventricular contractions (D_I); frequency of ventricular contractions 389/min.
- f. 108 min.: Early multifocal ventricular contractions (D_I) frequency of ventricular contractions 348/min.

After 110 min. extrasystoles disappeared, frequency became normal, while signs of ischaemia were maintained.

In three guinea-pigs the first electrocardiographic changes appeared 100–110 min. after the introduction of antigen with a gastric probe. Weak signs of ischaemia were noted only in D_{II} , with a maintained sinus rhythm. No significant changes of cardiac frequency. 5–10 minutes after the manifestation of

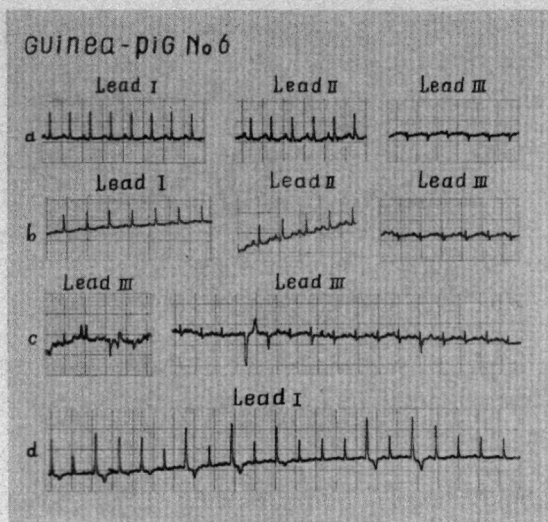


Fig. 6.

the first electrocardiographic changes early multifocal ventricular contractions appeared, as well as disorders of the WPW syndrome (Fig. 6).

In spite of the almost identical type of changes noted in the already analysed guinea-pigs the intensity of these changes was less.

Guinea-pig No. 6:

- a. Control before administration of antigen: sinus rhythm; frequency 290/min.
- b. 110 min.: Slight signs of ischaemia in D_I . No changes in D_{II} and D_{III} ; sinus rhythm; frequency 288/min.
- c. 115 min.: Early multifocal ventricular contractions (D_{III}); frequency of ventricular contractions 283/min.
- d. 120 min.: Changes according to the WPW syndrome (D_I); frequency of ventricular contractions 288/min.

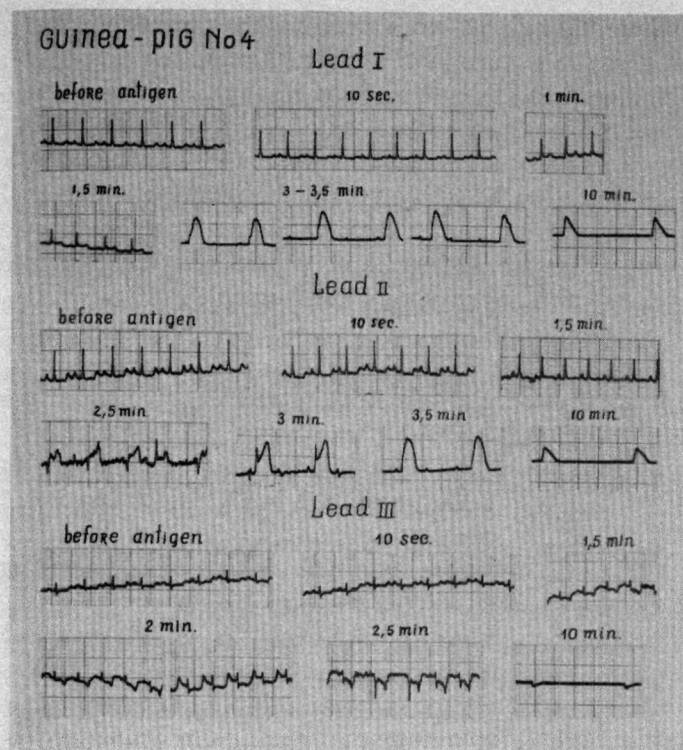


Fig. 7.

It should be pointed out that during electrocardiographic recordings, which lasted from 3.5 to 4 hours, no signs of anaphylactic reaction was noted. In 7 guinea-pigs in whom electrocardiographic changes were noted, the anaphylactic reaction of the isolated heart and intestine was positive. Positive results show that the animals were sensitized and that the dose of antigen administered with a gastric probe was only provocative and not attacking, as was the case in experiments carried out with isolated organs.

The intravenous injection of antigen (1 ml of 0.1 per cent solution) was carried out in 9 guinea-pigs (B_2). Four guinea-pigs manifested a typical clinical picture of anaphylactic shock with a lethal result 6-10 minutes later.

In these animals 10–20 sec. after the intravenous injection of antigen, the picture of the onset of ischaemia was visible in D_I . In the second minute signs of strongly manifested ischaemia appeared in all three leads with a maintained sinus rhythm, whilst cardiac frequency was increased. One minute later, with the manifestation of early ventricular contraction, complete atrioventricular block with dissociation of heart beats, as well as severe ischaemia, appeared. Premortal electrocardiographic changes appeared between 6 and 10 minutes (Fig. 7).

Guinea-pig No. 4.

- a. Control before administration of antigen: sinus rhythm; frequency 207/min.
- b. 10 sec.: First signs of ischaemia, slight lowering of T Wave voltage in D_I ; sinus rhythm; frequency 213/min.
- c. 1 min.: Severe ischaemia, rising of ST segment in D_I ; sinus rhythm, frequency 238/min.
- d. 1.5 min.: Signs of ischaemia in all three standard leads sinus rhythm; frequency 258/min.
- e. 2 min.: Severe ischaemia in D_{III} ; sinus rhythm; frequency 246/min.
- f. 3 min.: Complete atrioventricular block with dissociation of the heart beats (D_{II}); strongly pronounced ischaemia; frequency of auricles 254/min. and of ventricles 100/min.
- g. 3–3.5 min.: Complete atrioventricular block in D_I and D_{II} ; strong ischaemia; auricular frequency 227/min. and ventricular 94/min.
- h. 6–10 min.: Premortal electrocardiographic changes and death in the 12th minute.

The five remaining guinea-pigs in this group, in which no electrocardiographic changes were noted after intravenous injection of antigen and in which the anaphylactic reaction of the isolated heart and intestine was negative, proved that sensitization did not occur in them.

DISCUSSION

Clinicians have long noted that many alimentary allergic manifestations may be followed by functional cardiac disorders. It is known that in some patients stenocardiac attacks appear after eating meat, as these persons were sensitive to this kind of food. *Hansen* (11) stated in his book that some more serious functional cardiac disorders could be the consequence of alimentary sensitization. He pointed out that various forms of arrhythmias, tachycardia and cardiac block were recorded in some subjects after taking food to which they were sensitive.

Such data in the literature helped us to arrive at our own conception of the possibility of fixing of antibodies to the myocardium of experimental animals sensitized through the mucous membrane of the digestive tract.

There were contradictory opinions on the possibility of allergic sensitization through the mucous membrane of the digestive tract. *Djuričić* and others (5) found that guinea-pigs could be sensitized if concentrated ovalbumin solution or horse serum was introduced into stomach with a probe. These authors obtained anaphylactic sensitization with anaphylactic shock when the antigen, which was used for sensitization of animals, was intravenously administered, or was given through the stomach of the animal.

By analyzing the results obtained in the experimental group (A) it was clearly evident that there was a possibility of the formation of shock-tissue in the myocardium if the sensitization of animals was carried out through the mucous membrane of the digestive tract.

As the percentage of positive anaphylactic reactions of the isolated heart was lower when sensitization was carried out by the intraperitoneal route, it may be concluded that the mucous membrane of the intestine and the liver represent barriers, and that sensitization through the digestive tract appears only under certain conditions; these may be damage of the entero-hepatic barrier, or functional insufficiency of the excretory organs of the digestive tract.

In the other experimental group (B) we found that guinea-pigs sensitized through the digestive tract, in which an anaphylactic reaction was obtained by administration of antigen with a gastric probe, or intravenously 5-7 days after sensitization, manifested the electrocardiographic changes recorded *in vivo*. In 18 guinea-pigs of this group (B₁), after sensitization ovalbumin was introduced in the same way as sensitization was carried out (by the digestive tract). No animal showed visible signs of an anaphylactic reaction. But in 7 guinea-pigs electrocardiographic changes were noted. In 4 cases they appeared between 30 and 40 minutes after administration of the antigen, with signs of ischaemia and increase of cardiac frequency (D_I). Between 30 and 110 minutes the frequency increased, ischaemia became more severe and early ventricular contractions appeared (D_{II}). In the 106th minute a salvo of early ectopic contractions was noted (D_I). After 110 minutes extrasystoles disappeared, the frequency became normal, while ischaemia was maintained.

In three guinea-pigs with positive electrocardiographic changes the first signs of ischaemia appeared 100 minutes after the administration of antigen. The intensity of these changes was less and alterations were maintained for a shorter time.

In the second group of guinea-pigs which were sensitized in the same way, the repeated administration of antigen on the 7th and 10th day after sensitization was carried out intravenously. In 4 out of 9 guinea pigs in this group (B₂) the first electrocardiographic changes were recorded in three standard leads 10-20 sec. after the administration of antigen. The first signs of ischaemia appeared (as in group B₁) with an increased frequency, which was quickly replaced by bradycardia, early ventricular contractions, complete atrioventricular block and extreme bradycardia. Between 2 and 4 minutes, in condition of clinically developed anaphylactic shock typical premortal electrocardiographic changes of the dying heart were noted.

From these results it can be seen that the animals in which the repeated administration of antigen was carried out in the

same way as sensitization, *i.e.* by the digestive tract, survived. The others in group B₂ in which antigen was repeatedly introduced by intravenous route and which manifested electrocardiographic changes, anaphylactic shock had a lethal issue.

These results therefore indicate the role of the entero-hepatic barrier not only on the onset of sensitization, but also in the anaphylactic reactions. They show, however, that there is a possibility that even the mucous membrane of the digestive tract may induce not only sensitization of the organism and fixation of sessile antibodies to the myocardium, but also an anaphylactic reaction. This is not followed by visible clinical signs; it occurs only in some shock-tissue; it is associated with certain functional disorders of these organs; and it is transitory. According to the results of these experiments an anaphylactic reaction was provoked in guinea-pigs by the administration of antigen through the digestive tract in the same way as sensitization was obtained; it provoked functional cardiac changes, especially in the circulatory system, without a visible clinical picture of anaphylactic shock.

Finally we would like to emphasize that such experimental results bore out already noted observations that in some patients after partaking of a certain food, stenocardiac disorders, heart palpitations and other subjective cardiac disorders may appear, and that these changes represent, sensitization of the organism to this kind of food.

CONCLUSION

1. Guinea-pigs sensitized through the mucous membrane of the digestive tract, when the antigen solution was added to the perfusion solution, presented the following changes of isolated heart beats:

- a. Increased frequency with decrease or increase of cardiac contraction amplitudes.
- b. Dissociation of heart beats with separation of auricular from ventricular contractions, atrioventricular arrest, and extrasystoles of ventricular origin.

- c. After this period the heart regained its normal functioning.

On the basis of results obtained in this experimental group it can be concluded that sessile antibodies are also fixed to the myocardium during sensitization through the mucous membrane of the digestive tract.

2. In guinea-pigs sensitized through the mucous membrane of the digestive tract, if antigen is repeatedly introduced in the same way, or intravenously, the following electrocardiographic changes appeared:

- a. If antigen was re-administred with a gastric probe: late and transitory signs of ischaemia, early ectopic ventricular contractions and distirnaces as in the WPW syndrome, but without any clinical symptoms of general anaphylactic reaction.
- b. If antigen was re-administred by the intravenous route: sudden and short signs of ischaemia, at first as in the previous group and later with complete atrioventricular block, and extreme bradycardia in clinically developed anaphylactic shock with death.

On the basis of the results of this experimental group it can be concluded that some transitory cardiac disorders can be explained by anaphylactic reaction which occurs in the myocardium and its blood vessels, and without other clinically visible signs of general anaphylactic reaction.

3. Fixation of antibodies to the myocardium and its blood vessels is possible in sensitization through the mucous membrane of the digestive tract, but to a lesser degree than if carried out in other ways. In sensitization and anaphylaxis it would seem from the experiments that there is an entero-hepatic barrier.

SUMMARY

This experimental work deals with fixation of sessile antibodies to the myocardium of guinea-pigs in case of sensitiza-

tion of these animals carried out by the digestive tract: performance of anaphylactic reaction of isolated heart; recording *in vivo* of electrocardiographic changes of heart beats after re-administration of antigen by gastric probe or intravenously.

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