

THE EFFECTS OF ANAPHYLAXIS, AND OF HISTAMINE, UPON THE CORONARY ARTERIES IN THE ISOLATED HEART

BY E. COWLES ANDRUS, M.D., AND HERBERT B. WILCOX, JR.,* M.D.

(From the Cardiographic Laboratory, Department of Medicine, Johns Hopkins University and Hospital, Baltimore)

(Received for publication, December 29, 1938)

In a previous communication (15), certain manifestations of anaphylaxis in the isolated perfused heart of the guinea pig were described. These were: (a) transient increase in the rate and amplitude of contraction, (b) delay in auriculoventricular conduction and changes in the form of the ventricular complexes of the electrocardiogram, and (c) frequent development of ectopic arrhythmias. In addition to these effects, some of which had already been recorded by the electrocardiograph in the intact animal, an additional effect was reported: a striking *reduction* in the rate of flow through the coronary vessels.¹ The similarity of this reaction to the effect of histamine upon the same preparation was emphasized.

In view of certain differences among various species in the reaction of the coronary arteries to histamine, the authors have proceeded to a study of the anaphylactic reaction in the isolated hearts of cats, in which histamine regularly brings about coronary *dilatation* (8, 2).

Methods

Sensitization to horse serum was accomplished by the intraperitoneal or subcutaneous injection of three doses of 1 cc. each at intervals of 5 to 7 days. Of 14 cats so treated five were tested, from 4 to 10 weeks after the last sensitizing dose, by the intravascular injection of 2 cc. of serum. In one animal no reaction occurred. The remaining four survived prompt reactions characterized by rapid breathing, retching, vomiting and defecation, followed by prostration.

* Jacques Loeb Fellow in Medicine, Johns Hopkins University.

¹ Similar results have been observed in a small number of experiments with the hearts of sensitized rabbits.

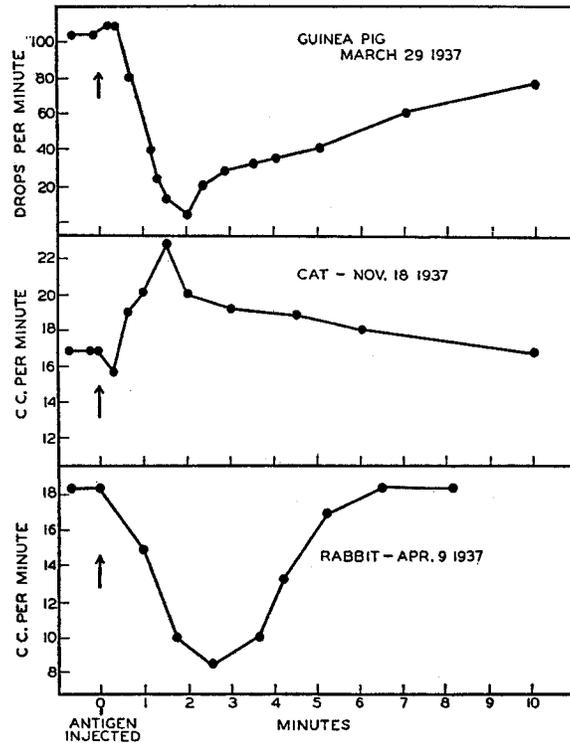
At the time of experiment the sensitized animals and the normal controls were killed instantly by a blow on the head and then their hearts were isolated and perfused with Ringer-Locke solution at 35°C. and a pressure of 75 mm. Hg, and the outflow from the coronary arteries collected and measured, according to the method previously described (15). The antigen, diluted to 1 cc. with warm Ringer-Locke's solution, was injected through the wall of the rubber tubing immediately above the cannula into the stream of the perfusing fluid.

The Results in Normal and Sensitized Animals

The exposure of the isolated heart of the normal cat to horse serum in the way described is not entirely without effect. Within 10 to 15 seconds after the injection the coronary flow suddenly diminishes, returning to its previous rate within 30 seconds. This reaction differs from the true anaphylactic response in at least two important particulars: (a) it is common to normal, sensitized and desensitized hearts alike, and (b) it fails to decrease, but rather tends to increase, in intensity with successive injections.² The duration of the effect corresponds more or less closely with the time required for the serum-containing fluid to pass through the heart. A series of 15 tests on four normal hearts produced no other discernible effect. Similar results were observed with desensitized organs.

In the hearts of sensitized cats, on the other hand, after the introduction of 0.01 to 0.1 cc. of serum, the early evanescent coronary constriction is followed by a striking *increase* in coronary flow, beginning about 1 minute after the injection and lasting from 2 to 5 minutes. With subsequent exposures to the antigen this latter effect diminishes in intensity or fails to appear. This conforms with a true anaphylactic reaction. The results of the first injections in ten experiments are summarized in Table I, showing increases of coronary flow of 18 to 48 per cent over the preinjection levels. This reaction is not accompanied by any uniform effect upon cardiac rate, which varies in the same experiments from an increase of 17 per cent to a decrease of 8 per cent of the rate before injection.

² The tendency of normal smooth muscle to undergo contraction upon the addition of serum has been observed in other organs suspended in Ringer's solution (12, 13, 4), in perfused blood vessels (3), in the coronary arteries of hearts previously perfused with Ringer's solution (3), and in the pulmonary vessels of normal and sensitized cats (6).



TEXT.-FIG. 1. The effect of anaphylaxis upon coronary flow in the isolated hearts of the guinea pig, the cat and the rabbit.

TABLE I

Changes in Cardiac Rate and Coronary Flow during Anaphylaxis in the Isolated Perfused Hearts of Cats

Date	Amount of serum injected	Cardiac rate change during reaction	Coronary flow increase during reaction
	cc.	per cent	per cent
Oct. 14	0.01	-8	35
" 26	0.01	+4	48
Nov. 10	0.01	0	22
" 11*	0.01	-5	30
" 18	0.01	+17	37
" 19*	0.01	0	18
" 29*	0.01	+5	35
Mar. 14	0.01	-7	48
" 15	0.01	-8.5	46
" 22	0.10	4	45

* Animals previously tested by intracardiac injection at 1, 4 and 5 weeks respectively. All three were demonstrably sensitive.

Text-fig. 1 contrasts the effect of anaphylaxis upon the coronary flow in the perfused heart of the cat with that in the heart of the guinea pig and rabbit. In each species these effects are qualitatively identical with the action of histamine upon the same preparation. The results, therefore, further support the view that, in the process of anaphylaxis, histamine or a similar substance is elaborated.

Anaphylaxis in the Hearts of Immune Animals

If repeated doses of horse serum, or other foreign protein, be given to a guinea pig, it may be demonstrated that the animal becomes refractory or "immune" to subsequent injections. These animals will then tolerate the intravenous administration of large amounts of the antigen without symptoms of anaphylaxis. But the isolated uterus of such an immunized guinea pig reacts no less than that of a sensitized animal upon the addition of small amounts of horse serum to its environment (4).

A series of comparable observations was conducted upon the hearts of guinea pigs. The animals were rendered resistant to the effects of horse serum by not less than 12 intraperitoneal injections of 0.1 cc. each—except as noted in Table II—and at intervals of 2 to 3 days. The degree of resistance so engendered was tested by the intravascular injection of serum *in vivo* 1 to 3 days after the last intraperitoneal dose. Under these conditions, four animals bore the intravascular administration of 0.2 cc. or 0.3 cc. of serum without symptoms, and three others 0.6 cc. or 0.8 cc. Three more survived mild reactions after the intravascular injection of 0.8 cc.; one of these tolerated the same dose 3 days later without symptoms. One animal (Dec. 6 *a*) died in typical shock upon the injection of 0.6 cc. of serum 3 days after the last intraperitoneal injection, although the other animal tested on the same day (Dec. 6 *b*) survived without symptoms.

As soon as it became apparent that the intravascular injection would not provoke symptoms, the hearts of seven animals were removed and perfused; two others were isolated 3 and 8 days respectively after the test *in vivo*; four more were tested by the method of perfusion only. In every instance the exposure of the isolated heart to 0.01 cc. of serum in the perfusate caused a typical anaphylactic reaction.

These results suggest anew that the immune guinea pig is protected

by an excess of circulating antibody. But in the heart isolated from such an animal, as in the isolated uterus, the quantity of fixed antibody is sufficient to provoke, upon combination with the appropriate antigen, an anaphylactic reaction indistinguishable from that of the heart of a sensitized animal.

TABLE II
Anaphylactic Reactions in Guinea Pigs Rendered Resistant to Horse Serum by Repeated Injections

Experiment	Number of doses* of horse serum	Intravascular serum <i>in vivo</i>		Isolated heart
		Amount	Reaction	Reaction to 0.01 cc. serum
		cc.		
Jan. 25 a	13	0.2	0	++++
" 26 a	13	0.2	0	++++
" 26 b	13	0.3	0	++
" 27 a	14	0.2	0	++++
Dec. 6 b	15	0.6	0	++++
Jan. 24 b	12	0.8	0	++++
†Dec. 7 b	13	0.6	0	Tested Dec. 15
Jan. 25 b	13	0.8	Mild, survived	Not tested
" 28 b	14	0.8	" "	+++
‡ " 25 c	13	0.8	" "	Tested Jan. 28
‡ " 28 a	14	0.8	0	+++
Dec. 6 a	15	0.6	Fatal	Not tested
" 7 a	16		Not tested	++++
† " 15	19		" "	++++
" 8	16		" "	++++
Jan. 24 a	12		" "	++++
" 27 b	14		" "	++++

* 0.1 cc. by intraperitoneal injection at intervals of 2 or 3 days.

† The same animal was used in these two experiments.

‡ The same animal was used in these two experiments.

Double Sensitization

Rosenau and Anderson (11) observed that guinea pigs could be rendered sensitive to three proteins at the same time and that, following a non-fatal reaction to one, such animals were desensitized to that alone. Dale (4) demonstrated multi-sensitization of uterine muscle of the guinea pig to horse serum, sheep serum and egg white, or even to separate serum proteins (Dale and Hartley, 5) but concluded that

desensitization to one antigen was not without effect upon the sensitiveness to the others.

The authors have tested the possibility of double sensitization of the heart in a small group of guinea pigs. Each animal received, by intraperitoneal injection, 0.1 cc. of horse serum and 0.1 cc. of a 10 per cent solution of egg albumen in Ringer-Locke solution. 4 weeks later, the isolated hearts of four of these guinea pigs reacted to each antigen in small amounts (Table III); after desensitization to one, a typical anaphylactic reaction followed exposure to the other. A fifth animal died in anaphylactic shock upon the injection of 0.4 cc. serum. Its heart, removed immediately and perfused, proved completely desensi-

TABLE III
Anaphylaxis in the Isolated, Perfused Hearts of Guinea Pigs Sensitized to Horse Serum and Egg Albumen

Date	First antigen	Reaction	Second antigen	Reaction
Nov. 30, 1937	Serum 0.01 cc.	+++	Albumen 0.1 cc.	++++
Dec. 3, 1937	" " "	++++	" " "	0
Jan. 10, 1938	" " "	++	" " "	++
" 12, 1938	" " "	++	" " "	++++
Nov. 30, 1937	Serum 0.40 cc.*	Fatal	" " "	++++†
Jan. 13, 1938	Albumen 0.10 cc.	+++	Serum 0.01 cc.	++++

Albumen administered in 1.0 per cent solution.

* Serum injected *in vivo*.

† Perfused heart.

tized to serum but quite sensitive to albumen. The heart of a sixth reacted on exposure to serum but not to albumen.

A larger series of observations would be required to determine, in this preparation, whether desensitization to one antigen may not exert a quantitative effect upon the sensitivity to another. But the few results recorded above seem sufficient to demonstrate that the heart of the guinea pig is susceptible to independent sensitization to two different antigens.

Anaphylaxis to a Bacterial Substance

The evidence establishing the occurrence of anaphylaxis induced by bacterial antigens includes reports of the demonstration of the typical

response of the isolated uterus of the sensitized guinea pig upon exposure to appropriate bacterial substances (14, 16). The type-specific polysaccharides derived from the pneumococcus by Heidelberger (9) are not, apparently, capable of bringing about active sensitization in the guinea pig but provoke the characteristic reaction in passively sensitized animals of this species (1) or in the isolated uteri thereof (10).

TABLE IV
Anaphylaxis in the Isolated, Perfused Hearts of Guinea Pigs upon Exposure to Pneumococcus Polysaccharide

Amount of anti-pneumococcus (Type I) rabbit serum injected intraperitoneally	Elapsed time before experiment	<i>In vivo</i> test in intact animal		Isolated heart on perfusion	
		Amount of pneumococcus (Type I) polysaccharide injected intravascularly	Reaction	Amount of polysaccharide I injected	Reaction
<i>cc.</i>	<i>days</i>	<i>mg.</i>		<i>mg.</i>	
0.5	2	0.5	Fatal	Not tested	
2	2	1	"	"	"
3	3	1	"	"	"
1	1	2	"	"	"
2	2	Not tested		2	++++
1	2	"	"	1	++++
2	2	"	"	1	++++
2	2	"	"	0.5	++++
1	2	"	"	0.001	++++
1	2	"	"	0.0001	++++
1	1	"	"	1	0
				2	0
3	3	"	"	0.1	0
				0.8	0
				2	0

The authors examined the response of the hearts of sensitized guinea pigs to the polysaccharide derived from the pneumococcus by Felton (7).³ Attempts to produce active sensitization to this type-specific substance proved entirely unsuccessful. Passive sensitization was accomplished in 12 animals by the intraperitoneal injection of Type I antipneumococcus rabbit serum. The intravenous injection into

³ The authors are indebted to Dr. Lloyd G. Felton who placed a quantity of this substance at their disposal.

four animals, 24 to 72 hours later, of 0.5 to 2.0 mg. of Type I polysaccharide (Felton), resulted, in each case, in fatal anaphylactic shock. The hearts of six of the remaining eight animals were removed and perfused 2 days after the original administration of antipneumococcus serum. Upon the addition of polysaccharide to the perfusate in amounts as small as 0.0001 mg. there ensued the characteristic anaphylactic response with consequent desensitization (Table IV). This reaction failed to appear in the hearts of the two other animals, isolated 24 hours and 72 hours respectively after the original dose of serum.

SUMMARY

Anaphylaxis in the isolated, perfused hearts of cats has been shown to be accompanied by a considerable, though transient, *increase* in coronary flow. This result is contrasted with that observed in the hearts of guinea pigs and rabbits in which the coronary arteries are constricted during anaphylaxis. Attention is directed to the fact that, in the hearts of these three species, the effects of anaphylaxis and of histamine are qualitatively parallel.

The characteristic anaphylactic response in the isolated hearts of guinea pigs has been evoked: (*a*) in the organs removed from immune animals, (*b*) by each of two antigens (horse serum and egg albumen) under conditions of double sensitization, and (*c*) upon exposure of the hearts of passively sensitized animals to the type-specific polysaccharide of the pneumococcus.

It is evident that, among the effects of anaphylaxis upon smooth muscle in various organs, there must be considered that upon the coronary arteries.

BIBLIOGRAPHY

1. Avery, O. T., and Tillett, W. S., *J. Exp. Med.*, 1929, **49**, 251.
2. Cruikshank, E. W. W., and Rau, A. S., *J. Physiol.*, 1927, **64**, 65.
3. Cushny, A. R., and Gunn, J. A., *J. Pharmacol. and Exp. Therap.*, 1913, **5**, 1.
4. Dale, H. H., *J. Pharmacol. and Exp. Therap.*, 1913, **4**, 167.
5. Dale, H. H., and Hartley, P., *Biochem. J.*, 1916, **10**, 408.
6. Drinker, C. K., and Bronfenbrenner, J., *J. Immunol.*, 1924, **9**, 387.
7. Felton, L. G., Kauffmann, G., and Stahl, H. J., *J. Bact.*, 1935, **29**, 149.
8. Gunn, J. A., *J. Pharmacol. and Exp. Therap.*, 1926, **29**, 325.
9. Heidelberg, M., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1925, **42**, 727.

10. Mehlmann, J., and Seegal, B. C., *J. Immunol.*, 1934, **26**, 1.
11. Rosenau, M. J., and Anderson, J. F., *J. Infect. Dis.*, 1907, **4**, 552.
12. Schultz, W. H., *J. Pharmacol. and Exp. Therap.*, 1911, **3**, 299.
13. Stewart, G. N., *J. Exp. Med.*, 1912, **15**, 547.
14. Tomcsik, J., and Kurotchkin, T. J., *J. Exp. Med.*, 1928, **47**, 379.
15. Wilcox, H. B., Jr., and Andrus, E. C., *J. Exp. Med.*, 1938, **67**, 169.
16. Zinsser, H., and Mallory, T., *J. Immunol.*, 1924, **9**, 75.