constriction recorded in the fully dilated specimen seemed to correspond closely in size, shape and timing to the notching of the curve of dilation as in Fig. 2. None of the human coronary arteries ever assumed any significant degree of tone. Variations in dosages of test drugs employed varied the magnitude and duration of the reaction produced; it never reversed the response to a given drug (as from constriction to dilation or vice versa). Acetylcholine in varying doses uniformly produced constriction of the coronary arteries examined. Histamine likewise produced only vasoconstriction of the coronary arteries examined; this effect was uniform regardless of variation in the dosage employed (Fig. 3). It is felt that this technic will prove of value in assessing the effect of drugs and hormones upon the coronary and other arteries and that different responses may be obtained in vessels from subjects suffering from different pathological conditions.

Summary. 1. The method of employing angioplethysmokymography to evaluate the responses of coronary arteries to several drugs is described.

2. Epinephrine did not produce a uniform response in the coronary arteries examined. The relation of this inconsistency to the state of vasodilation of the specimen artery is discussed.

3. Acetylcholine was found to produce consistent coronary vasoconstriction in man, pig and sheep.

4. Histamine uniformly produces vasconstriction of the coronary arteries of man, pig, ox and sheep.

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Release of Histamine During Hemolytic Reactions in the Blood of Rabbits. (17710)

HADDON M. CARRYER AND CHARLES F. CODE.

From the Division of Medicine and Section on Physiology, Mayo Foundation and Mayo Clinic, Rochester, Minn.

It has been shown in different species that histamine is released from the cells of one or several organs during anaphylactic reactions(1-6). Special circumstances pertain in the blood. Most of the histamine contained in blood is held in the white cell layer which separates between the plasma and the red cells when unclotted blood is centrifuged(7,8). This bound or fixed histamine is released by comparatively mild trauma such as clotting or agitation. During anaphylactic reactions in drawn blood, Katz has found that histamine is released from the cells into the plasma(9). In Katz's experiments, rabbits were sensitized by several intravenous injections of egg white. Later when the antigen was added *in vitro* to heparinized samples of the blood the histamine content of the plasma increased 100 to 600% above that of plasma of control samples of the same blood to which no antigen had been added. Studies by Dragstedt and his co-workers(10,11) and Rose and Browne(12) have confirmed and

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extended these findings. Dragstedt, Ramirez de Arellano, Lawton and Youmans(13) have demonstrated the liberation of histamine from the blood cells of passively sensitized rabbits when the appropriate antigen was added *in vitro* to the blood. Katz and Cohen(14) extended their observations from rabbits to studies of the human being. They found that histamine was released from the blood cells of patients with ragweed sensitivity when an extract of ragweed pollen was added to the blood *in vitro*. Such a release did not occur when the extract was added to the blood of normal subjects or when extracts of other pollens were added to the blood of patients sensitive to ragweed.

This study was undertaken to determine whether or not hemolytic reactions in drawn blood would release histamine from the cellular elements into the plasma.

Methods. Rabbits were used throughout this investigation because the histamine content of their blood is high. This makes it especially suitable for *in vitro* studies. Hemolytic reactions were produced by the addition of sheep erythrocytes to the drawn blood of animals previously sensitized to this antigen. The erythrocytes were obtained by drawing blood from sheep into a solution of sodium citrate to make a final concentration of 0.25% sodium citrate. After centrifugation and discarding the plasma, the red cells were washed by centrifuging three times with a physiologic solution of sodium chloride. The rabbits were sensitized to the washed sheep cells by 4 intravenous injections 1.0 cc of a 50% suspension of the cells given at 4-day intervals. Experiments were conducted no sooner than one week following the final injection of sheep erythrocytes. This method of sensitizing the rabbit to sheep erythrocytes is a modification of that used by Kolmer(15).

To minimize trauma and contact with foreign surfaces, blood was obtained directly from the cut end of a carotid artery. The rabbits were anesthetized with pentobarbital sodium. The carotid artery was dissected free from surrounding tissues in the neck and a small hemostat was placed on branches of the vessel. The artery was then sectioned and the attached hemostat was used to direct the spurt of blood from the artery into a vessel previously coated with paraffin wax. Blood samples were kept in an unclotted state by the addition of heparin.

Histamine analyses were made according to the Code modification of the Barsoum and Gaddum method(16). In the experiments in which plasma histamine was determined, it became apparent that contact of blood even with clean glassware during separation of the plasma was often accompanied by a considerable release of histamine from the formed elements of the blood. Great care was then exercised to avoid undue trauma to the blood by the use of paraffin-coated glassware and by directing the spurting arterial flow down the side of the collecting tube so that no foaming occurred, and by minimizing agitation of the blood. With these precautions only very small quantities of histamine were found in control samples of plasma.

Determinations of antisheep erythrocyte hemolysin titer in the plasma or serum of the rabbit were made according to the method described by Kolmer. In the majority of the experiments, 10 cc samples of rabbit blood were placed in paraffin-coated centrifuge tubes. One cc of physiologic salt solution, or physiologic salt solution in which was suspended washed sheep erythrocytes in a concentration of 50% by volume, were added to equal samples of the blood. After incubation for one hour at 37°C, the samples were centrifuged and the supernatant fluid analyzed for its histamine content. Many of the tests were carried out with duplicate 10 cc samples of blood.

Results. Tests were performed on 11 sensitized rabbits. The histamine content of the

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TABLE I.
Increase in Histamine Content of Plasma During *in vitro* Hemolytic Reactions in Rabbit Blood.

			In vitro study	
			μg histamine/cc plasma in	
			Control sample:	Test sample:
Rabbit	Blood hemolysin titer	Min of incubation	Blood + 1 cc saline sol.	Blood + 1 cc saline sol. containing sheep erythrocytes
1	*	60	.32	.60
2	*	60	.67	1.28
3†	1:5,000	60	.07	.80
			.07	.70
4†	1:10,000	60	.09	1.14
			.08	1.22
5†	1:10,000	Nil	.03	1.33
6†	1:2,500	60	.13	.28
			.13	
7†	1:10,000	30	.20	1.25
			.33	1.14
8†	1:50,000	30	.33	1.09
			.38	2.00
9†	1:25,000	30	.25	1.25
			.09	
10†	1:50,000	30	.75	1.20
			.71	
11†	1:50,000	30	.13	1.02
			.11	1.13

* Hemolysin titer not determined, but extensive hemolysis occurred upon addition of sheep erythrocytes.
† Paraffined glassware used in test.

plasma in which a hemolytic reaction had occurred was consistently increased being usually 2 to 15 times that of control samples (Table I). In one test carried out without incubation, the release of histamine was apparently completed within the time required for separation of the plasma (about 5 minutes, Table I, rabbit 5). A 50% suspension of sheep erythrocytes such as was used to evoke the hemolytic reactions was found to contain less than 0.03 μg of histamine per cubic centimeter. The histamine released during the reaction was therefore not derived from the sheep erythrocytes.

As a means of testing whether or not sheep plasma elements adhering to the erythrocytes were participating in the reactions in rabbit blood the physiologic salt solution used in the final washing of the sheep erythrocytes was tested as an antigen with blood obtained from the sensitized rabbits. Heparinized blood of 2 rabbits sensitized to sheep erythrocytes was divided into a series of equal samples. To 4 of these, physiologic salt solution was added. To another 4, samples of the physiologic salt solution used in the final washing of sheep erythrocytes was added, and to a third group of samples washed sheep erythrocytes were added. All samples were incubated for one hour at 37°C. There was no significant release of histamine into the plasma of the blood to which had been added either pure physiologic salt solution or the physiologic salt solution last used in the washing of the sheep erythrocytes (Table II). As in the other tests, there was, however, a pronounced rise in the plasma histamine in those blood samples to which washed sheep erythrocytes had been added. It is concluded that the release of histamine occurring during the hemolytic reaction was not brought about by elements of sheep plasma adhering to the erythrocytes.

Comment. The results obtained indicate that in the rabbit histamine is released from the formed elements of the blood during hemolytic reactions. Loss of histamine from the cells of the blood was not, however, directly checked in this study. It seems unlikely that

TABLE II.
Concentration of Histamine in Plasma of Rabbit Blood After *in vitro* Additions of Sheep Erythrocytes, Saline Solution Washings of the Erythrocytes, and Physiologic Salt.

	μg histamine/cc plasma of blood to which had been added:		
Rabbit	0.5 cc of physiologic salt sol.	0.5 cc of final washing sol.	0.5 cc of 50% suspension of washed sheep erythrocytes
12	.25	.20	1.25
	.09	.21	
13	.13	.33*	1.02
	.11	.36*	1.13

* Blood shaken to effect adequate mixing.

histamine was produced by the reaction and much more probable that its distribution was altered through a liberation from the cells of the blood. If this was the case, the plasma histamine values following the *in vitro* hemolytic reactions were so high that the histamine could not have been derived from the red cells alone but must have come in the main from the elements of the buffy coat. This participation of the white cells and platelets in the hemolytic reaction was further indicated by the reduction in the size of the buffy coat layer in those samples in which hemolytic reactions occurred.

The mechanism by which the hemolytic reaction releases histamine from the formed elements of the blood is not known. A release of histamine into the plasma of drawn blood takes place after such simple trauma as contact of the blood with glass or with fragments of debris or by clotting or shaking of the blood. The release of histamine in the course of the hemolytic reaction is most likely the result of some form of chemical or physicochemical trauma.

The results of this study indicate that release of histamine from blood cells into plasma may contribute to the symptoms observed during hemolytic transfusion reactions.

Summary. Rabbits were sensitized by the repeated intravenous injection of washed sheep erythrocytes. After an appropriate period, addition of the washed sheep red cells to the rabbit blood *in vitro* produced prompt hemolysis. This *in vitro* hemolytic reaction in the rabbit blood was always accompanied by an increase in the histamine content of the plasma.

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The Influence of the Spleen on Hematopoietic Recovery After Irradiation Injury.* (17711)

L. O. JACOBSON, E. L. SIMMONS, W. F. BETHARD, E. K. MARKS, AND M. J. ROBSON.
From the Biology Division of the Argonne National Laboratory, and the Department of Medicine and the Institute of Radiobiology and Biophysics of The University of Chicago.

It has been reported by Jacobson and associates(1,2) that if the spleens of mice are mobilized surgically and protected with lead during whole body exposure to a dose of 600

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