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Effect of Blood Volume Changes on Renin-Like Activity in Blood. (29370)

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Although renin secretion is said to be increased in shock(3,4,7,10), no exact data have been presented either on the degree or onset of the rise. It was assumed that, by its effect on the blood pressure, renin might counteract severe hypotension(10). On the other hand, despite various hypotheses(13, 14) the mechanism of renin release is still not defined. Employing isovolemic cross circulation in the rat, with a nephrectomized animal highly sensitive to renin as indicator, we found a very constant concentration of a renin-like substance in the blood of intact animals(6,11). This method is suitable to determine variations in the concentration of renin-like activity in blood under various experimental conditions. To obtain more precise information on a possible role of the renin-angiotensin system in the regulation of homeostasis we investigated the effects of changes in the intravascular volume on renin secretion in rats. The present paper reports on the influence of acute blood loss and of forced blood transfusion on the concentration of renin-like substance in blood as well as on the renin content of the kidneys.

Material and methods. Isovolemic cross circulation between the donor rat and the indicator animal, nephrectomized about 20 hours previously, was established through the femoral vessels according to a previously described technique(6,11).

a. *Hemorrhage experiments.* White male rats of average weight 260 g were anesthetized with urethane (1.4 g/kg body weight). Blood pressure was recorded in the left carotid artery by means of a mercury manometer. After connection of the femoral vessels with those of the nephrectomized indicator animal but before starting cross circulation, 5 ml of blood were taken from the femoral artery of the donor rat within a 5-minute period. Blood pressure fell rapidly to values between 20 and 30 mm Hg but increased gradually during 10 minutes up to 30 to 40 mm Hg. Ten to 15 minutes after bleeding, cross circulation was established for 10 minutes. Following disconnection of the animals, the blood pressure of the indicator rat was recorded for another 10 to 15 minutes in order to see whether it remained elevated as occurs in nephrectomized rats after injection of renin. At the end of the experiment the donor animals were killed by intravenous injection of air, the kidneys were removed, and their renin content was determined as described previously(2).

In another series of experiments the donor rats were nephrectomized 5 to 10 minutes before bleeding. From these animals also, 5 ml of blood were removed and then cross circulation installed. Since, under these conditions, blood pressure recovered more slowly, cross-circulation flow in the femoral artery was less than half (0.8 ml/min) of that in the non-nephrectomized rats (2.0 ml/min). Earlier experiments had demonstrated that such a reduction of flow rate does not influence the concentration of renin-like substance in blood(11). Altogether 25 to 30 minutes elapsed between nephrectomy and the start of cross circulation.

b. *Overtransfusion experiments.* White male rats of average weight 250 g received 3 infusions of 4 ml each of heparinized (0.3 ml of a 0.5% heparin solution) rat blood which immediately before had been drawn from the carotid artery of a male rat, anes-

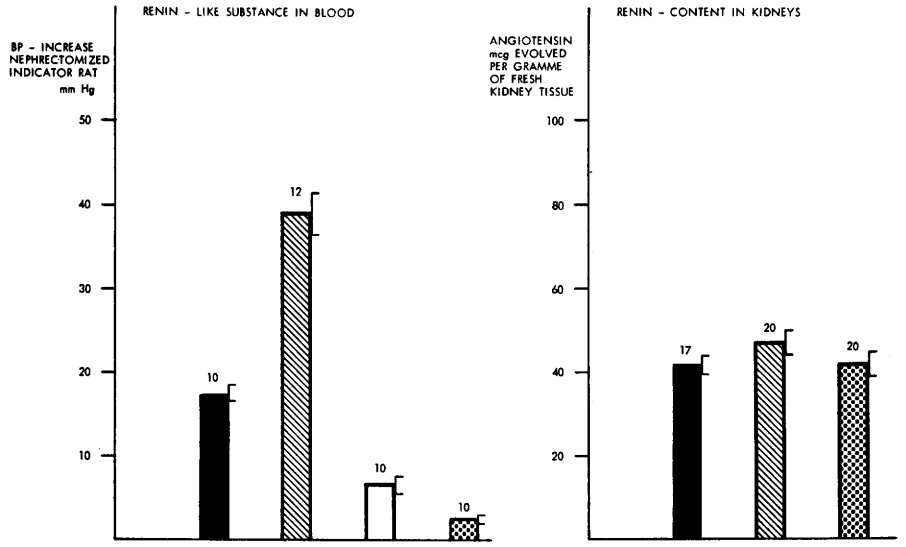

FIG. 1. Renin-like activity in blood and renin concentrations in kidneys of rats after severe hemorrhage and after overtransfusion compared to values in control rats. Numbers of experimental animals (left side of figure) or of kidneys bioassayed (right side of figure) are shown at top of columns. Decrease in renin-like substance after nephrectomy and hemorrhage was the same as after nephrectomy not followed by bleeding.

black columns: normal control rats
hatched columns: intact rats } 10 to 15 min after hemorrhage
white columns: nephrectomized rats } (5 ml per rat)
stippled columns: overtransfused rats after transfusion of 3 times 4 ml blood within 24 hr

thetized with urethane. The blood was infused into one of the tail veins. The second infusion of 4 ml was given 3 hours, and the third 20 hours after the first. Four hours later (24 hours after the first infusion), cross circulation between the overtransfused animal and a nephrectomized indicator was established. In another group of animals, cross circulation was not installed until one week after the last transfusion.

As in the hemorrhage experiments, the donor animals were killed after a cross-circulation period of 10 minutes, and the kidneys were removed for determination of their renin content. Renin was determined as angiotensin according to the method previously described, and the content was expressed as γ angiotensin/g fresh renal tissue (2,9).

Results. a. *Hemorrhage experiments.* Cross circulation with an intact donor animal led, in the nephrectomized indicator rat, to a blood-pressure increase of 17.5 $\pm$ 1.1 mm Hg. The renin content of the kidneys expressed as angiotensin was 41.4 $\pm$ 2.3 γ/g fresh renal tissue (Fig. 1). Both values correspond very well to those obtained in former experiments(6,11).

When donor animals were connected to indicator rats within 10 to 15 minutes (average 12.8 min) after hemorrhage, the blood-pressure increase in the indicator animals was about twice as high as during cross circulation with an intact rat, reaching a mean value of 39 $\pm$ 2.6 mm Hg. According to former experiments, this corresponds to a 3- to 4-fold increase of the renin concentration in the blood of donor rats(12). The renin content of the kidneys, however, was about the same as in intact animals, the average value found being 46.9 $\pm$ 3.1 γ angiotensin/g fresh renal tissue (Fig. 1).

To determine whether the blood-pressure increase in the indicator rats was produced by a substance of renal origin, bleeding experiments were repeated with nephrectomized rats. In these animals, immediately after hemorrhage, systemic blood pressure fell to about the same level of 20 mm Hg but had less tendency to increase afterwards.

In only 3 out of 10 rats were values of 30 mm Hg reached within 10 to 15 minutes, and in one animal the pressure was only 25 mm Hg. As a consequence the flow during cross circulation was less than half of that in experiments with non-nephrectomized donors. Under these conditions only a slight increase in blood pressure (6.6 ± 1.1 mm Hg) occurs in the recipient animals, indicating that the increased reaction elicited by donors with intact kidneys is due to a humoral factor of renal origin. The residual pressor activity may be explained by the fact that disappearance of renin after nephrectomy takes about one hour, whereas cross circulation was installed in about half that time(6,12).

b. *Transfusion experiments.* When overtransfused animals were used as donors, there was only a minimum increase of blood pressure or none at all in the indicator rats when cross circulation was established. Thus no renin-like substance remains in the blood of rats with extensively increased intravascular volume. On the other hand, the renin content of the kidneys of the overtransfused indicator rats was within the normal range (Fig. 1). In preliminary experiments in which cross transfusion was initiated 1 to 2.5 hours after transfusion of 5 to 9 ml of blood, no clear influence on the concentration of renin-like substance in blood was demonstrable. It can therefore be stated that the renin-like substance present under normal conditions in the blood disappears when the intravascular volume has been maintained elevated for about 20 hours.

When, however, one week had elapsed between overtransfusion and the cross-transfusion experiment, an average blood-pressure increase of 12.3 ± 4.5 mm Hg was obtained in the indicator animals. This finding may be interpreted as a partial return towards normal values of the circulating renin-like substance. The renin content of the kidneys was again found to be within the normal range (46.6 ± 8.5 γ angiotensin/g renal tissue). The data are summarized in Table I.

TABLE I

Condition of donor rat	Donor		Indicator body wt, g	Flow between the 2 animals, ml/min	Indicator BP increase, mm Hg	Donor. Renin content of kidneys, γ angiotensin/g fresh tissue
	BP before cross circul., mm Hg	Body wt, g				
Intact (10)	115.0 ± 2.5	262.0 ± 7.5	257.0 ± 6.0	2.0 ± .09	17.5 ± 1.1	41.4 ± 2.3
Bled (12)	113.0 ± 2.8	255.0 ± 5.4	262.0 ± 3.7	2.0 ± .12	39.0 ± 2.6	46.9 ± 3.1
Bled and nephrex (10)	111.0 ± 2.7	264.5 ± 2.5	260.5 ± 3.0	.8 ± .06	6.6 ± 1.1	41.4 ± 3.1*
Overtransfused (10)†	110.5 ± 2.5	248.7 ± 1.4				
	113.5 ± 5.0	247.2 ± 1.4	250.8 ± 2.5	1.3 ± .06	2.5 ± .5	42.0 ± 2.5
1 wk after overtransfusion (6)‡	106.6 ± 3.3	253.0 ± 1.8				
	106.0 ± 2.0	264.1 ± 11.0	250.0 ± 3.3	1.4 ± .06	12.3 ± 4.5	46.6 ± 8.5

* Determined in excised kidneys.
† Upper row: before transfusion; lower row: after transfusion.
‡ Upper row: before transfusion; lower row: one wk after transfusion.

Discussion. By means of the isovolemic cross-circulation technique it was possible to demonstrate that changes in intravascular volume were accompanied by variations in concentration of renin-like substance in blood. While marked acute reduction of blood volume, provoked by hemorrhage, results in a 3- to 4-fold increase which was evident within 10 to 15 minutes of bleeding, augmentation of intravascular blood volume was followed by an almost complete disappearance of renin-like substance. Although there is no definite proof that the blood-pressure increase of the nephrectomized indi-

cator animal is due to renin, there is much evidence in favor of this assumption(11), in particular the following: (a) Only the nephrectomized animal, highly sensitive to renin, reacts to cross circulation with a blood-pressure increase which in its course is very similar to that which follows injection of exogenous renin; (b) there is a strong parallelism in chronic experiments between concentration of renin-like activity in blood and content of renin in the kidneys; (c) there is a lack of response by the indicator animal when cross circulated with a nephrectomized animal.

The results of our bleeding experiments are in agreement with previous observations (7), which, however, are not quantitative, and with those reported recently by Paladini and Scornik(8). The latter found in dogs, within one hour after severe bleeding, a steep rise in the blood-angiotensin concentration which increased during subsequent hours when blood was not retransfused. Although we were unable to obtain a definite reduction in renin-like activity after blood transfusion of normovolemic rats within the same short period as that within which there was an increase after hemorrhage, renin-like substance disappeared from the blood when the intravascular volume was maintained elevated for about 24 hours. It is not surprising that the response of the kidney by secretion of renin is more prompt after hemorrhage than after a corresponding increase of the circulating blood volume. If we consider that renin-angiotensin either by itself or by stimulating aldosterone secretion is involved in the homeostatic regulation of the intravascular volume, the probability of a sudden reduction occurring because of blood loss is much greater than the contrary.

Neither the decrease nor the increase in renin-like activity in blood was accompanied by a detectable change in renin content of the kidneys. This is not surprising when the short period between hemorrhage and the moment when cross circulation is initiated is considered. On the other hand, the stimulus of an increase in blood volume during 24 hours is not sufficient to influence the renin content of the kidney. As no pure renin is available so far, a quantitative relationship between renal and circulating renin cannot definitely be established. In cross circulation experiments with angiotensin we found that 0.3 γ/kg/min had to be infused into a nephrectomized donor rat in order to obtain a blood pressure increase of about 30 mm Hg in the indicator animal (*i.e.* 3 γ angiotensin for the 10-minute period of cross circulation) (6). As intact kidneys contain an equivalent of 40 γ angiotensin/g renal tissue (average kidney weight being 915.6 $\pm$ 34.8 mg(9), the total amount of renin circulating in the blood is only a small fraction of the renin store in the kidney. Hence it may be assumed that the intact kidney should easily meet an acute need by either increaseing or decreasing the output of renin. On the other hand, in chronic experiments such as sodium load or sodium depletion as well as in certain forms of experimental renal hypertension, variations in circulating renin are accompanied by parallel alterations of renal renin content(6,11).

The response of renin secretion to hemorrhage is closely parallel to the observed increase in aldosterone secretion(1) and in the antidiuretic activity in blood(5). Hence it must be considered that a triad of humoral factors are involved in the homeostatic regulation of intravascular volume.

Summary. By means of the isovolemic cross-circulation technique rapid variations in concentration of renin-like activity in blood were demonstrated following marked alterations of the intravascular volume. Bleeding leads within 10 to 15 minutes to a 3- to 4-fold increase in concentration of renin-like substance in the blood, while overtransfusion results in a reduction of this activity within 24 hours. Nephrectomized rats do not react to hemorrhage with an increase in renin-like activity in blood. The renin content of the kidneys showed no detectable changes in bleeding as well as in overtransfusion experiments.

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Serum Manganese in Myocardial Infarction.* (29371)

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That the serum manganese concentration rises following myocardial infarction was reported by Hedge, Griffith, and Butt(1), who concluded that this increase is more useful diagnostically than the elevation in serum glutamic-oxalacetic transaminase activity (SGOT). The present study was undertaken to determine the specificity of increased serum Mn for myocardial damage and to determine whether degree of serum Mn elevation is proportional to extent of damage.

Materials and methods. Serum was obtained from normal persons and from hospital patients with myocardial infarction or other disease.

Samples were also taken from dogs before and after the production of acute coronary occlusion. Adult mongrel dogs were anesthetized with morphine and nembutal and were given artificial respiration by intermittent positive pressure. An incision was made through the fourth intercostal space, the pericardium entered, and silk nooses placed loosely around the anterior descending branch of the left coronary artery 5-10 mm distal to the origin of the circumflex artery. The lumen of the artery was progressively narrowed until complete occlusion was effected after 20 minutes; the obstructing ligature was left intact. The pneumothorax was reduced by over-inflation of the lungs as the chest was closed. Electrocardiographic tracings and venous blood samples were taken before operation and at intervals following the occlusion. Samples of normal and infarcted cardiac tissue were removed at necropsy for manganese determinations. All infarctions were confirmed by sectioning and microscopic study.

Sera were dry ashed, with the aid of a little nitric acid, as suggested by Thiers(2) and analyzed by the method of Gates and Ellis(3). Attempts to simplify the method by using trichloroacetic acid precipitation instead of ashing were unsuccessful, whether or not (ethylenedinitrilo) tetraacetic acid was added. The use of ion exchange resin to remove Mn from serum and recover it by elution was also disappointing.

SGOT determinations were made by the method of Cabaud *et al*(4) (normal is below 40 units).

Results. The data from the dogs are recorded in Table I. Before operation the serum Mn averaged 3.7 μg% with a range of

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