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# LIBERATION OF HISTAMINE DURING REACTIVE HYPERAEMIA AND MUSCLE CONTRACTION IN MAN

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Barsoum & Gaddum [1935*a*], working on dogs, found that the histamine equivalent of venous blood collected from a limb during reactive hyperaemia increases above normal; it reaches a maximum immediately on release of the circulation after which it rapidly declines. Anrep & Barsoum [1935] obtained a similar effect by severely restricting the arterial blood supply to the gastrocnemius muscle without completely arresting the circulation. Barsoum & Smirk [1936], repeating these experiments on man, observed a two- to four-fold increase of the histamine concentration in the plasma after a 10–20 min. obstruction of the circulation of the arm, the histamine content of the blood corpuscles remaining in most experiments unaltered. The authors conclude that liberation of a histamine-like substance during circulatory arrest accounts at least in part for the hyperaemia. Marcou, Comsa & Chiriceano [1937] found a small increase of histamine in venous blood during the first minute after release of an arterial obstruction of a limb in man.

Recently, Kwiatkowski [1941], also working on man, failed to obtain any evidence of histamine release during reactive hyperaemia; he found no change in the histamine content either of the plasma or of the corpuscles. In view of the importance of this problem for the understanding of the nature of reactive hyperaemia we undertook to repeat these experiments with the object of finding the cause of the conflicting results obtained by the previous observers.

## Methods

Our experiments were made on normal subjects or on patients suffering from minor surgical disabilities. The preparation of the blood extracts and the estimation of histamine were made by the method of Barsoum & Gaddum [1935*b*]. To separate the plasma, the blood was either heparinized or collected in paraffined syringes and centrifuged in cooled paraffined tubes.

The subject was made to rest for about half an hour before the experiment. After withdrawing one or two samples of venous blood from one arm, to

serve as a control, a sphygmomanometer cuff was placed round the other arm and inflated to above the systolic pressure for 10–20 min. The needle of the syringe was inserted into the vein a few seconds *before* the release of the circulation, and the first blood sample was collected as exactly as possible between the 5th and 30th sec. after the release. In many experiments a second and a third sample was collected between the 60th and 90th and between the 120th and 150th sec. respectively.

Most previous observers collected the blood samples after releasing the pressure in the cuff to zero. Kwiatkowski, in some experiments, released it to 40 or 60 mm. Hg. This was done in order to prevent the blood from leaving the limb during the period of reactive hyperaemia and to avoid the excessive dilution of the blood which had been in contact with the tissues during the circulatory arrest. Since no evidence was provided that this object was achieved we decided to investigate in preliminary experiments on animals the effect of venous compression upon the extent and duration of the reactive hyperaemia.

*Effect of partial venous compression upon the reactive hyperaemia.* The experiments were made on dogs anaesthetized with chloralose and injected with chloralose-fast pink (0·1 g./kg.) to prevent coagulation. The femoral artery and vein were dissected for a length of about 3 cm., and a steel band 0·5 cm. in width was passed round the leg under the blood vessels. A cannula was inserted into the peripheral end of the femoral vein after which the steel band was tightened so as to obstruct all the collateral communications. The emerging blood was made to pass through an artificial resistance of the type used in the heart-lung preparation and to enter a reservoir the top of which was connected with a volume recorder. The reservoir was periodically emptied of blood which was then reinjected into the animal. A manometer inserted between the artificial resistance and the leg registered the venous pressure, while the arterial pressure was measured with a mercury manometer which was adapted to record also the maximum-minimum pressures. The records so obtained did not represent the systolic and diastolic pressures of the animal, but the maximal pressure variations including those caused by the respiration.

In order to study the effect which a rise of venous pressure has on the reactive hyperaemia, the resistance on the venous outflow was varied in the different experiments between zero and a pressure midway between the maximal and the minimal arterial blood pressure. The artery was compressed for 10 min. and the venous resistance was adjusted to any desired height about 1 min. before the release of the circulation. Care was taken to prevent the cooling of the limb during the arrest of the circulation. The blood flow during the period of hyperaemia was compared with the resting blood flow as measured with a completely free venous outflow before the artery was compressed. After the decompression of the artery the venous resistance was

maintained for not more than 3 min. Only those experiments were considered in which occlusion of the femoral artery led to a complete cessation of the outflow of blood from the vein, showing absence of collateral connexions. The arterial compressions were repeated at intervals of 10–15 min. after the return of the blood flow to normal. The results of a typical experiment are given in Table 1.

TABLE 1. Dog. 9·5 kg. Duration of each arterial compression, 10 min. The maximal-minimal arterial blood pressure was measured shortly before the compression of the artery

| Maximal-minimal arterial blood pressure in mm. Hg | Maximal venous pressure in mm. Hg | Normal blood flow in c.c. per 30 sec. | Blood flow during reactive hyperaemia in c.c. in consecutive periods of 30 sec. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 |
| 145/118 | 0 | 12·0 | 79·6 | 56·1 | 36·2 | 26·5 | 19·9 | 15·8 |
| 145/118 | 64 | 11·0 | 67·1 | 40·7 | 27·0 | 19·5 | 15·2 | 14·5 |
| 140/116 | 86 | 10·5 | 51·3 | 35·8 | 22·6 | 17·1 | 13·2 | 11·2 |
| 135/116 | 106 | 10·5 | 28·0 | 19·6 | 16·6 | 13·8 | 9·4 | 5·7 |
| 132/112 | 120 | 10·0 | 12·9 | 10·8 | 7·6 | 5·1 | 4·2 | 3·6 |

The effect of increased venous resistance upon the hyperaemia was less than expected. Thus, with venous pressures 118, 54, 30 and 10 mm. Hg below the minimal arterial blood pressure the blood flow during the first 30 sec. of the hyperaemia was 6·6, 6·0, 5·0 and 2·7 times respectively above the normal. The increase of the blood flow was abolished only by raising the venous pressure above the minimal arterial pressure.

On applying these results to man, in order to prevent the blood flow from increasing during the initial stages of the hyperaemia above the resting level, the venous pressure should be raised above the diastolic pressure of the subject. Venous pressures of 40–60 mm. Hg, as used by Kwiatkowski, would not be sufficient to prevent the blood from leaving the limb. In view of this conclusion two series of experiments were performed on the human subject. In the first, the blood samples were collected after complete decompression of the sphygmomanometer; in the second, the collection was made after releasing the cuff to a pressure slightly below the systolic pressure.

## RESULTS

### *Observations with complete and partial decompression*

The experiments with complete decompression were made on five subjects. The venous blood was collected between the 5th and 30th sec. after release of the sphygmomanometer, while the control blood samples were taken from the other arm either during or shortly before the period of arterial compression. The results of ten observations are given in Table 2.

It can be seen from Table 2 that the histamine equivalent of the plasma collected immediately after the release of the circulation is in every experiment increased above that of the normal plasma. In a few experiments the

TABLE 2. Showing the effect of 20 min. arterial compression on the histamine equivalent of the plasma and corpuscles of the venous blood collected between the 5th and 30th sec. after complete release of the sphygmomanometer. The histamine equivalent is given in $\mu$g. of histamine acid phosphate per c.c.

| | Histamine equivalent of venous blood in $\mu$g./c.c. | | | |
|---|---|---|---|---|
| | Normal | | Reactive hyperaemia | |
| Subject | Plasma | Corpuscles | Plasma | Corpuscles |
| 1 | 0·025 | 0·13 | 0·036 | 0·12 |
| 1 | 0·022 | 0·12 | 0·032 | 0·11 |
| 2 | 0·005 | 0·10 | 0·010 | 0·09 |
| 2 | 0·007 | 0·08 | 0·013 | 0·09 |
| 3 | 0·015 | 0·13 | 0·020 | 0·13 |
| 3 | 0·014 | 0·12 | 0·022 | 0·12 |
| 4 | 0·009 | 0·14 | 0·013 | 0·16 |
| 4* | 0·007 | 0·11 | 0·015 | 0·13 |
| 5 | 0·008 | 0·13 | 0·013 | 0·14 |
| 5* | 0·008 | 0·14 | 0·016 | 0·15 |

* The pressure in the cuff was released in the experiment on subject 4 to 40 mm. and in subject 5 to 60 mm. Hg.

histamine concentration is as much as doubled, in most the increase is less. The results of the two observations in which the cuff was released to 40 and 60 mm. Hg respectively do not differ from the rest.

The experiments with partial decompression were made on four subjects, two of whom had served in the preceding series of observations. After the subject had been allowed to rest and his blood pressure had been measured, his arm was compressed for 10 or 20 min. At the end of the compression the cuff was released to a pressure slightly below the systolic pressure and the blood samples were collected as before between the 5th and 30th sec. after the release. The results of eight observations are given in Table 3.

TABLE 3. Showing the effect of arterial compression on the histamine equivalent of the plasma and corpuscles of the venous blood collected between the 5th and 30th sec. after the release of the sphygmomanometer to a pressure slightly below the systolic pressure of the subject. The histamine equivalent is given in $\mu$g. of histamine acid phosphate per c.c. The normal blood samples were collected with a free venous blood flow

| | | | | Histamine equivalent of venous blood in $\mu$g./c.c. | | | |
|---|---|---|---|---|---|---|---|
| | | | | Normal | | Reactive hyperaemia | |
| Subject | Blood pressure in mm. Hg | Pressure in cuff at time of collection in mm. Hg | Duration of compression in min. | Plasma | Corpuscles | Plasma | Corpuscles |
| 3 | 130/ 85 | 120 | 20 | 0·015 | 0·14 | 0·070 | 0·16 |
| 3 | 130/ 82 | 120 | 20 | 0·013 | 0·19 | 0·066 | 0·18 |
| 5 | 125/ 72 | 100 | 20 | 0·007 | 0·14 | 0·028 | 0·16 |
| 5 | 120/ 70 | 100 | 20 | 0·008 | 0·12 | 0·032 | 0·12 |
| 6 | 150/100 | 140 | 10 | 0·011 | 0·21 | 0·022 | 0·23 |
| 6 | 145/ 95 | 130 | 10 | 0·018 | 0·24 | 0·033 | 0·24 |
| 7 | 115/ 70 | 95 | 10 | 0·013 | 0·07 | 0·020 | 0·08 |
| 7 | 115/ 70 | 100 | 20 | 0·015 | 0·10 | 0·067 | 0·10 |

It can be seen on comparing Tables 2 and 3 that the increase in the histamine equivalent of the plasma collected with partial decompression after a 10 min.

circulatory arrest is approximately the same as that of the plasma collected with a free venous outflow after a 20 min. compression of the artery. The plasma histamine of the blood collected with partial decompression after 20 min. of arterial occlusion is increased 4–5 times above normal; with complete decompression the increase was less than double. No change in the histamine concentration of the corpuscles was found in the experiments with partial or complete decompression.

*The duration of the increased liberation of histamine*

In the preceding experiments the blood samples were taken immediately after the end of the arterial occlusion. In order to determine the length of time during which the plasma histamine remains increased above normal, second and third blood samples were collected 1 and 2 min. respectively after the release of the circulation. The results of the experiments performed on four subjects are given in Table 4. Two observations were made on each subject at an interval of a few days; in the first, the blood was collected with complete and, in the second, with partial decompression of the arm.

TABLE 4. Histamine equivalent of the plasma collected at different intervals of time after complete and after partial decompression of the arm. The arterial occlusion was in every experiment 20 min. The histamine equivalent of the corpuscles is omitted from the table as presenting no points of interest. The normal blood samples were collected with a free venous blood flow

| Subject | Blood pressure in mm. Hg | Pressure in cuff at time of collection in mm. Hg | Histamine equivalent of plasma in μg./c.c. | | | |
|---|---|---|---|---|---|---|
| | | | Normal | Reactive hyperaemia 5–30 sec. | Reactive hyperaemia 60–90 sec. | Reactive hyperaemia 120–150 sec. |
| 1 | — | 0 | 0·024 | — | 0·022 | 0·022 |
| | 125/85 | 115 | 0·022 | — | 0·040 | 0·032 |
| 2 | — | 0 | 0·005 | 0·009 | 0·005 | 0·005 |
| | 115/70 | 105 | 0·005 | 0·029 | 0·012 | 0·008 |
| 3 | — | 0 | 0·012 | 0·018 | — | 0·011 |
| | 135/85 | 125 | 0·009 | 0·048 | 0·018 | — |
| 7 | — | 0 | 0·015 | 0·028 | 0·016 | — |
| | 120/75 | 105 | 0·013 | 0·055 | — | 0·017 |

A comparison of the results obtained on each subject shows that with a free circulation no increase in the plasma histamine can be detected in samples collected 1 min. after decompression of the arm. On the other hand, when the venous outflow is restricted the plasma histamine remains above normal for as long as 2 min. after the release of the artery. Similar restrictions of the venous blood flow for 3–4 min. not preceded by a period of arterial occlusion cause no change in the histamine concentration of the plasma.

It follows from these experiments that the histamine produced by the tissues during the period of ischaemia is rapidly washed out unless the blood flow is greatly restricted. With a free circulation the plasma histamine returns to normal long before the end of the hyperaemia. This suggests that the excess

histamine appearing in the blood diffuses from the tissues into the capillaries during the period of the circulatory arrest and that probably no further diffusion takes place after the re-establishment of the circulation.

*Liberation of histamine during muscular contraction*

Having obtained evidence of histamine release during reactive hyperaemia in man we proceeded to investigate if a similar release can be detected in the human subject during muscular contractions. The experiments were made with the same technique as those on the reactive hyperaemia, except that the arrest of the circulation in the arm was continued for only 2 min. The sphygmomanometer cuff was inflated to above the systolic pressure and the subject was told to make fifteen vigorous contractions of his hand and forearm at the rate of one contraction each 2 sec. The contractions were timed to begin on the 60th sec. of the arterial compression and to end 30 sec. later. At the end of the 2 min., i.e. 30 sec. after the last contraction, the pressure in the cuff was released to slightly below the systolic pressure of the subject, and the venous sample was collected in the usual way between the 5th and 30th sec. after the decompression of the artery. The same procedure was followed in the control experiments, which were usually performed on another day, except that the arm remained completely relaxed. The results obtained on two subjects are given in Table 5.

TABLE 5. Showing the effect of arterial compression for 2 min. with the arm at complete rest and with fifteen vigorous contractions made between the 60th and 90th sec. of compression. The normal blood samples were collected with a free venous blood flow

| Subject | Blood pressure in mm. Hg | Pressure in cuff at time of collection in mm. Hg | Histamine equivalent in μg./c.c. | | | |
|---|---|---|---|---|---|---|
| | | | Normal | | Active | |
| | | | Plasma | Corpuscles | Plasma | Corpuscles |
| 3 | 130/ 90 | 120 | 0·015 | 0·140 | 0·013 | 0·140 |
| arm at rest | 125/ 82 | 115 | 0·012 | 0·120 | 0·014 | 0·120 |
| 3 | 140/105 | 130 | 0·014 | 0·140 | 0·060 | 0·130 |
| 15 contractions | 130/ 90 | 120 | 0·012 | 0·130 | 0·085 | 0·140 |
| 8 | 108/ 68 | 95 | 0·010 | 0·025 | 0·011 | 0·022 |
| arm at rest | 110/ 70 | 95 | 0·009 | 0·029 | 0·010 | 0·029 |
| 8 | 110/ 70 | 95 | 0·012 | 0·030 | 0·075 | 0·070 |
| 15 contractions | 110/ 70 | 95 | 0·010 | 0·028 | 0·050 | 0·060 |

It can be seen from Table 5 that when the arm is at rest occlusions of the circulation for 2 min. were insufficient to bring about a detectable change in the histamine equivalent of the venous blood. Similar occlusions of the circulation during which fifteen contractions of the muscles were made by the subject led to a conspicuous increase of the histamine equivalent of the plasma in subject 3, and of the plasma and corpuscles in subject 8. The increase was even greater than that which follows occlusion of the artery for 20 min. with the arm at rest.

Subject 8 presents a special interest as being the only subject in whom the corpuscular histamine increased above normal in most experiments with muscular contraction and arterial occlusion. An explanation of this apparent exception is provided by the observations of Anrep, Barsoum, Talaat & Wieninger [1939*a*], who showed that histamine added to shed blood is not taken up by the corpuscles unless its concentration in the plasma exceeds that of the corpuscles. When this occurs the histamine becomes equally distributed between plasma and corpuscles. Similar conditions obtained in subject 8. His corpuscular histamine was so low that after muscular contractions the plasma histamine increased above that of the corpuscles. As in experiments *in vitro* the excess histamine became equally distributed between the two phases of the blood.

Our observations are in accord with the results obtained on animals which show that the increase of the blood flow [Lewis & Grant, 1926] and the increase of the histamine concentration in the venous blood are much more conspicuous during muscular contractions than after occlusion of the artery [Anrep, Barsoum, Talaat & Wieninger, 1939*b*].

### *Effect of venous congestion*

Reactive hyperaemia is evoked not only by a temporary occlusion of the artery but also by prolonged venous congestion. With the latter, the hyperaemia is shorter and less conspicuous than that following a complete arrest of the circulation of equal duration. The cause of the hyperaemia is undoubtedly the same in both conditions, namely, a reduction or arrest of the blood supply, the changes in the venous pressure being only of incidental interest [Lewis & Grant, 1926]. Anrep & Barsoum [1935] found that diminution of the blood flow in a muscle to about half of the normal is accompanied by a steady liberation of small amounts of histamine. Table 6 shows that venous congestion, if considerable, evokes a liberation of histamine also in the human subject.

TABLE 6. The sphygmomanometer cuff was inflated to below the diastolic pressure of the subject. The blood samples were taken after 20 min. of compression without releasing the cuff. The two experiments on each subject were made with an interval of a few days. The subjects were the same as in the experiments given in Table 5. The normal blood samples were collected with a free venous blood flow

| | | | Histamine equivalent in $\mu$g./c.c. | | | |
|---|---|---|---|---|---|---|
| | | | Normal | | Venous congestion | |
| Subject | Blood pressure in mm. Hg | Pressure in cuff in mm. Hg | Plasma | Corpuscles | Plasma | Corpuscles |
| 3 | 130/90 | 50 | 0·007 | 0·120 | 0·009 | 0·130 |
| | 135/95 | 90 | 0·009 | 0·100 | 0·028 | 0·110 |
| 8 | 110/70 | 40 | 0·009 | 0·029 | 0·011 | 0·029 |
| | 115/75 | 70 | 0·012 | 0·024 | 0·036 | 0·040 |

A 20 min. compression of the arm with a pressure 30–40 mm. Hg below the diastolic pressure of the subject caused no obvious change in the histamine concentration of the blood. On the other hand, when the compressing pressure was raised almost to the height of the diastolic pressure, the histamine of the plasma increased in both subjects about three times above the normal: As in experiments with muscular contractions the histamine equivalent of the corpuscles of subject 8 increased, to become equal to that of the plasma. Venous congestion of short duration had no detectable effect on the histamine of the blood.

## Discussion

The experiments described in this communication confirm and extend our previous observations made on animals. In the human subject, as in the dog, conditions accompanied by a relatively deficient circulation lead to a liberation of measurable amounts of histamine. During the large increase of the blood flow which follows a period of ischaemia the histamine containing blood is greatly diluted with fresh blood and is rapidly washed out of the limb, the histamine equivalent returning to normal within the first minute of the reactive hyperaemia.

The increase in the histamine equivalent of the blood is rendered more conspicuous when the blood flow, during the period of the hyperaemia, is prevented from increasing above normal by compressing the veins with a pressure above the diastolic pressure of the subject. Venous compression with 40–60 mm. Hg, as used by Kwiatkowski, is insufficient since it does not greatly affect the blood flow during the initial stage of the hyperaemia. When the blood flow through the arm is controlled, an increase in the histamine of the plasma can be found as late as 2 min. after the onset of the hyperaemia.

The failure of Kwiatkowski's experiments to demonstrate a liberation of histamine during reactive hyperaemia was probably due to an insufficient control of the blood flow through the arm and to the collection of the blood samples at a time when the liberation of histamine had already ceased.

Vigorous muscular contraction of short duration is accompanied in man, as in the dog, by a release of histamine which is considerably larger than that following arterial occlusion of the same duration. There is no reason to suppose that the cause of histamine liberation by the contracting muscle is different from that operating during arterial occlusion or venous congestion. In all these conditions there is a relative local deficiency of circulation. The liberated histamine must be considered as one of the factors which readjust the balance between the metabolic requirements of the tissues and their blood supply.

SUMMARY

1. Muscular contraction, arterial occlusion and prolonged venous congestion in the human subject are accompanied by a release of histamine by the tissues.

2. The release of histamine is rendered more conspicuous by controlling the circulation during the period of the hyperaemia by raising the venous pressure above the diastolic pressure of the subject.

3. In most subjects the histamine increases only in the plasma; in subjects with a low histamine equivalent of the corpuscles the histamine increases in plasma and corpuscles.

4. The reasons of Kwiatkowski's failure to demonstrate a liberation of histamine during reactive hyperaemia are discussed.

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