Estimated regional blood flow by rubidium 86 distribution during arousal from hibernation

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Bullard, Robert W., and Gordon E. Funkhouser. Estimated regional blood flow by rubidium 86 distribution during arousal from hibernation. Am. J. Physiol. 203(2): 266-270. 1962.—The local organ or tissue blood flows during the process of arousal from hibernation have been estimated in the 13-lined ground squirrel by the Sapirstein method, which consists of the measurement of the regional distribution of injected rubidium 86. The studies demonstrated that during arousal there is a confinement of blood flow to the thoracic regions. After the heart rate has attained 100 beats/min, blood flow increases to the anterior portions of the animal. At the arousal level characterized by a heart rate of 200 beats/min, blood flow to anterior and thoracic tissue had attained levels almost equal to control flows. Posterior tissue flows were still much lower than control flows. The centralization of blood flow to thoracic and anterior tissues did not occur in the rat in the hypothermic state.

A characteristic feature of the process of arousal from the hibernation state appears to be the restriction of blood flow to peripheral and posterior regions (1). The circulatory confinement to the chest and to anterior portions, including the brain, has been demonstrated in the hamster by X-ray studies of the distribution of injected Thorotrast (2) and by differential temperature measurements in several species (1-3). Neither of these two methods possesses high enough resolution to yield quantitative information on the changes in organ blood flow during arousal.

Sapirstein (4) has successfully utilized the measurement of the fractional distribution of intravenously injected rubidium 86 to estimate regional blood flows in the rat. It was our purpose to utilize this technique to estimate the organ and tissue blood flow changes at different stages of arousal of the 13-lined ground squirrel.

Four physiological states as characterized by the heart rate were selected for study. Specifically these were: 1) deep hibernation, heart rates less than 25 beats/min; 2) partial arousal, heart rates of 100 beats/min; 3) partial arousal, heart rates of 200 beats/min; and 4) normothermic controls, heart rates of 300-360 beats/min. Regional blood flows were determined in 16 squirrels, 4 in each of the preceding states.

The basis of the method of organ or regional blood flow estimation we used is as follows: when a substance, such as rubidium, that readily distributes into the process. Since completion of our studies a report by Johansen (5) has appeared in which fractional distribution of injected rubidium 86 was similarly measured in the arousing and awake arctic ground squirrel (Spermophilus undulatus).

METHODS
Thirteen-lined ground squirrels (Citellus tridecemlineatus) were allowed to hibernate in a cold room at 5 C. After an animal was observed to be in deep hibernation, it was taken from the cold room and a polyethylene catheter (PE 50) was chronically implanted in the jugular vein according to the method described by Popovic and Popovic (6). No anesthetic other than the hibernation dormancy was used. The squirrels recovered promptly and were returned to the cold room or kept for control experiments. After the complete recovery or subsequent hibernation, which usually occurred in 3-4 days, the experimental procedure was started. Subdermal safety-pin electrodes for electrocardiographic recording and 27-gauge copper-constantan thermocouples for colonic and esophageal or thoracic temperature recording were inserted. This procedure initiated the arousal process, which was studied at an ambient temperature of 5 to 7 C until the desired level of arousal was attained; then the blood flow estimation was made. For studying changes in later arousal the catheterized animals were restrained by being taped to boards until the desired arousal level was attained. In control studies fully recovered catheterized animals were briefly restrained for the heart rate determination and injection procedure described below and then immediately sacrificed.

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The basis of the method of organ or regional blood flow estimation we used is as follows: when a substance, such as rubidium, that readily distributes into the
in intracellular compartment is injected into the circula-
 tion, the fraction of the total amount deposited in a
tissue will be proportional to that fraction of the total
blood flow, or of the cardiac output, reaching that
tissue. The amount of substance deposited will remain
in that tissue for a certain time period. According to
Sapirstein (4) the tissue content of Rb\(^{86}\) remained
nearly constant in the normothermic rat from 16 sec
to 1 min after injection. The animals in the present
study possessed a wide range of temperature and cardiac
outputs; it was decided therefore, to standardize the
procedure by allowing the heart to beat 200 times after
the injection, before the circulation was halted and
tissues assayed for radioactivity.

When the electrocardiograph (ECG) indicated that
arousal had progressed to the desired level 10-20 \(\mu\)C
of Rb\(^{86}\) in 0.05-0.21 ml of saline was injected through
the implanted venous catheter. The animals were
stunned with a blow on the head after 200 heartbeats
had passed, the thorax was opened, and the heart
clamped. To obtain the data in deep hibernation the
injections were made with the least possible disturbance.
After the injection the thermocouples and ECG
electrodes were inserted and the above procedures
followed.

After the circulation was stopped, selected organs and
tissue areas were removed, weighed to 0.01 g, and
digested in a measured amount of strong KOH solution
on a steam bath. After digestion, 1 ml of the solution
was dried on a planchet and radioactivity was measured
with a shielded Geiger-Müller tube and decade scaler
(model DS-1A, Nuclear Measurements Corp.). The
radioactivity was then corrected to counts per organ.
By appropriate calculations the fraction of the total
injected activity in each organ was determined.

It was not technically feasible to measure simultane-
ously cardiac output with the Rb\(^{86}\) distribution measure-
ments. Cardiac output was determined in another series
of experiments by the dye dilution method described
in an earlier paper (7). The average stroke volume during
arousal was found to be 0.15 ml with a range of 0.12–0.24
ml in ten determinations. Stroke volume appeared to
show no consistent change with a change in thoracic
temperature or heart rate. Cardiac output in the present
study was calculated by multiplying the measured heart
rate times the average stroke volume of 0.15 ml. Organ
or local blood flows were then estimated by the relation-
ship:

\[
\text{cardiac output (ml/min)} \times \frac{\text{organ activity}}{\text{total injected activity}} = \text{organ blood flow rate (ml/min)}
\]

The organ blood flow was then corrected to milliliters
per gram of tissue per minute.

For orientation purposes, deep colonic temperatures,
cesophageal temperature, and heart rate were studied in
another series of experiments on 18 ground squirrels
during the complete arousal process at an ambient
temperature of 5°C.

The organ and tissue blood flows were similarly studied
in rats (Harlan strain) at three physiological states:
1) normothermic controls, 2) hypothermia with a heart
rate of 200 beats/min, and 3) hypothermia with a heart
rate of 100 beats/min. The general experimental pro-
dure and the cardiac output values for the rat used here
are presented in a previous paper (7).

RESULTS

The time required for complete arousal at an ambient
temperature of 5–7°C ranged from 1.0 to 7.2 hr after the
initiating disturbance. The mean arousal time in the 18
ground squirrels in this series was 3.6 hr. As shown in
Fig. 1, during arousal heart rate increased with an
increase in thoracic temperature. The relationship,
however, was only approximate as spurious variations
in heart rate occurred that did not appear to be tempera-
ture dependent. Deep colonic temperatures (thermo-
couple inserted 4 cm) lagged behind thoracic tempera-
tures until the completion of the arousal process in all
animals studied.

The average percentages of the total injected radio-
activity that appeared in each tissue in four different
physiological states are shown in Table 1. In those
squirrels classified as deeply hibernating the highest
fractions or percentages of Rb\(^{86}\) were found in heart,
brown fat, diaphragm, kidney, and liver, in that order.
In animals that had progressed to an arousal state
characterized by a heart rate of 100 beats/min the
fraction of total isotope deposited increased in thoracic
structures and axillary brown fat. The fraction found in
both anterior and posterior and in peripheral tissues
showed a decrease. At an arousal state of 200 heartbeats/
min an increased fraction was found in anterior tissue
such as head and forelimb. Tissues posterior to the
diaphragm had low rubidium uptake at this arousal

\[
\text{FIG. 1. Relationship of heart rate and deep colonic temperature to thoracic temperature during the arousal process at an ambient temperature of 5°C. Height of vertical lines at each point represents standard deviation. Each curve is the mean of 18 determinations.}
\]
forelimb

spleen

heart

diaphragm

tail

Table 1. Percentage of total injected activity deposited per gram of tissue during arousal from hibernation

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Deep Hibernation</th>
<th>Arousal, 100 beats/min</th>
<th>Arousal, 200 beats/min</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Icart</td>
<td>8.44 (6.93-11.26)</td>
<td>9.49 (7.32-12.80)</td>
<td>10.73 (10.28-10.96)</td>
<td>4.47 (3.90-5.19)</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.43 (1.20-1.18)</td>
<td>0.31 (0.10-0.49)</td>
<td>0.31 (0.28-0.51)</td>
<td>6.84 (6.01-7.51)</td>
</tr>
<tr>
<td>Liver</td>
<td>1.08 (0.71-1.80)</td>
<td>0.58 (0.43-0.80)</td>
<td>0.58 (0.50-0.66)</td>
<td>1.50 (1.06-1.81)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.19 (0.50-1.90)</td>
<td>0.29 (0.22-0.55)</td>
<td>0.29 (0.15-0.98)</td>
<td>0.78 (0.38-1.15)</td>
</tr>
<tr>
<td>Gut</td>
<td>0.40 (0.30-0.48)</td>
<td>0.20 (0.10-0.37)</td>
<td>0.07 (0.02-0.12)</td>
<td>0.84 (0.47-1.16)</td>
</tr>
<tr>
<td>Testes</td>
<td>0.18</td>
<td>0.18</td>
<td>0.06 (0.06-0.07)</td>
<td>0.10</td>
</tr>
<tr>
<td>Forelimb</td>
<td>0.41 (0.21-0.59)</td>
<td>0.21 (0.10-0.27)</td>
<td>0.08 (0.06-0.14)</td>
<td>0.43 (0.41-0.46)</td>
</tr>
<tr>
<td>Ribs section</td>
<td>1.02 (0.69-1.30)</td>
<td>1.34 (1.22-1.41)</td>
<td>1.50 (1.07-1.92)</td>
<td>1.13 (1.03-1.30)</td>
</tr>
<tr>
<td>Pelvic section</td>
<td>0.44 (0.28-0.61)</td>
<td>0.22 (0.05-0.28)</td>
<td>0.04 (0.03-0.06)</td>
<td>0.04 (0.01-0.06)</td>
</tr>
<tr>
<td>Upper hind leg</td>
<td>0.22 (0.10-0.29)</td>
<td>0.05 (0.02-0.08)</td>
<td>0.08 (0.05-0.14)</td>
<td>0.12 (0.06-0.17)</td>
</tr>
<tr>
<td>Rear foot</td>
<td>0.19 (0.05-0.53)</td>
<td>0.08 (0.04-0.11)</td>
<td>0.04 (0.01-0.07)</td>
<td>0.22 (0.20-0.24)</td>
</tr>
<tr>
<td>Skin, upper back</td>
<td>0.20 (0.13-0.56)</td>
<td>0.06 (0.02-0.07)</td>
<td>0.02 (0.00-0.06)</td>
<td>0.17 (0.13-0.23)</td>
</tr>
<tr>
<td>Skin, lower back</td>
<td>0.11 (0.05-0.25)</td>
<td>0.07 (0.03-0.07)</td>
<td>0.03 (0.01-0.06)</td>
<td>0.11 (0.09-0.15)</td>
</tr>
<tr>
<td>Tail</td>
<td>0.33 (0.16-0.59)</td>
<td>0.07 (0.05-0.08)</td>
<td>0.04 (0.05-0.07)</td>
<td>0.02 (0.01-0.07)</td>
</tr>
<tr>
<td>Head</td>
<td>0.72 (0.52-0.59)</td>
<td>0.49 (0.24-0.64)</td>
<td>0.04 (0.05-0.07)</td>
<td>0.43 (0.32-0.51)</td>
</tr>
<tr>
<td>Axillary brown fat</td>
<td>3.88 (2.05-4.22)</td>
<td>5.95</td>
<td>4.20 (3.54-4.73)</td>
<td>2.84 (2.51-3.03)</td>
</tr>
<tr>
<td>Thoracic brown fat</td>
<td>4.03</td>
<td>4.49</td>
<td>3.19 (3.56-3.83)</td>
<td>2.50 (2.16-3.51)</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>3.14 (2.05-4.22)</td>
<td>5.17 (2.43-8.72)</td>
<td>5.51 (2.29-7.79)</td>
<td>1.72 (1.50-1.92)</td>
</tr>
</tbody>
</table>

Values are means, with ranges in parentheses. *Indicates less than 4 samples assayed.

State. Most control fractions, especially in posterior tissues, were more similar to those of animals in deep hibernation than those of the two arousal states. However, the fraction deposited in kidneys in deep hibernation and in arousal was much lower than that in control experiments in which kidney uptake was higher than any other tissue.

The over-all deposition of Rb-86 is more widespread in deep hibernation and fully awake controls. In early arousal deposition is limited to the thorax and in later arousal, to the thorax and anterior regions.

From the fraction of the total injected isotope deposited in each tissue and from the estimated cardiac output the blood flow to each tissue could be calculated. A mapping of tissue blood flow is shown in Fig. 2. In this blood flow calculation a mean stroke volume of 15 ml was multiplied by the measured heart rate for the cardiac output determination. Changes from this mean stroke volume would not alter the profile of the plotted curves (Fig 2) or the proportional relationship of flow in the different tissues, but it would simply shift the curves upward or downward.

The deeply hibernating ground squirrels all had heart heart rates of less than 25 beats/min when the circulation was stopped for the tissue assay. The regional mapping (Fig 2) indicates that brown fat, heart (coronary), and diaphragm have the highest blood flows in this state. Peripheral flows, although extremely low, were maintained. Of the four animals, probably only one remained in true deep hibernation from injection to killing time. This animal had slightly lower brown fat and coronary blood flows than the other three.

The most prominent change undergone when arousal had progressed to a level of 100 heartbeats/min was a marked increase in flow to brown fat, heart, and ribs. The rib section consisted of bone and muscle and can be contrasted with the pelvic section, which is also of bone and muscle. In general, flow outside of the thorax did not increase greatly with the exceptions of axillary brown fat and, to a lesser extent, head and liver.

With arousal that had progressed to a level of 200 heartbeats/min further flow increases occurred in thoracic tissues. Flow to the anterior portion (head and forelimb) likewise increased. At this state of arousal the blood flow to thoracic and anterior regions had increased to almost control levels. Liver, kidney, and pelvis showed some increase; but, in general, posterior blood flow was similar to that of the deep hibernators.

The increment in cardiac output obtained between the arousal state characterized by 200 heartbeats/min and the fully awake control state was delivered almost entirely to the regions posterior to diaphragm. Kidney, liver, stomach and intestine, and the pelvis all had much higher blood flows in the control state than in early arousal.

A similar mapping of regional blood flows for the rat in the normothermic state and at two levels of hypothermia yielded different results, as shown in Fig. 3. In most tissues regional flow was roughly proportional to the cardiac output at the three different states. A marked difference in flow between anterior, thoracic, and posterior flows was not obtained. However, in hypothermia a marked shutdown of flow occurred in peripheral tissues such as the tail, hind foot, hind limb, and forelimb.

Comment

From an extensive search of the literature it was concluded that the determination of the distribution of injected Rb-86 would be an ideal method of estimating tissue blood flows in small mammals (4). However, we had considerable doubt as to the effectiveness of the method in the temperature conditions of our experiments because of possible Q10 effects (8). To test the effectiveness, hypothermic ground squirrels at various temperatures were injected with Rb-86 in the descending aorta just anterior to the renal arteries. The changes in radioactivity of the posterior region of the squirrel were then studied, using a collimated scintillation probe and rate meter. At all temperatures the activity increased,
plateaued, and then gradually diminished. The difference in temperature had no detectable effect, except that the plateau was prolonged with cooling. The change of radioactivity at the anterior end of the animal was also studied after injecting into the descending aorta.

It was concluded from these experiments that the majority of the isotope reaching the tissue in the first circulation is deposited in that tissue at all temperatures. However, with time rubidium 86 would be distributed throughout the animal. In the experiments the fraction of total isotope deposited in the tissue was not temperature dependent, as the colder animals (the hibernators) had greater deposition in cool peripheral regions than did animals during arousal. This is readily apparent from Table 1. There was not a marked difference in distribution of the isotope with varying temperature gradients between the thorax and colon in the early stages of arousal, when these gradients were just beginning to develop. It is our conclusion that the fraction of the isotope taken up by the tissues is dependent on blood flow and not on temperature.

It is difficult to compare quantitatively the results of our studies with the rubidium distribution studies reported by Johansen (5), as he used a different species and obtained no heart rate data. Arousal was studied only at one level (oral temperatures equal to 30°C), which represented a later arousal stage than any we studied. His conclusions, however, are quite similar to those of this study. In arousal a high fraction of rubidium 86 was deposited in the thoracic tissues, anterior tissues, and brown fat. The results, however, were not extended to actual blood flow estimations.

The heart rate and temperature data of our study are essentially in agreement with the findings of other workers (1). The posterior restriction of blood flow in our study likewise agrees with the Thorotrast studies (2) in the arousing hamster and the local temperature studies (3) in the arousing 13-lined ground squirrels. In our experiments blood flow remained low and constant at peripheral regions for a considerable portion of arousal. In the anterior regions the vessels opened up before a heart rate of 200 beats/min was attained. Examination of the ground squirrel data of Adolph and Richmond (3) indicates that brain temperature and presumably blood flow began to rise rapidly when thoracic temperature was about 15°C. According to our data this would occur just after the heart rate had surpassed 100 beats/min. Lyman and O'Brien (9) have reported that in the arousal process a decrease in peripheral resistance occurs simultaneously with a rising blood pressure. Our studies indicate that this may be due to marked vasodilation occurring in the thoracic regions. Examination of the relationship of rectal temperature to heart rate suggests that the posterior blood flow increased with heart rates between 200 and 300 beats/min. Landau and Dawe (10) have emphasized the importance of respiratory activity in arousal and have pointed out that at certain stages respiratory activity may increase more rapidly than heart rate. Our blood flow measurements also emphasize the role of respiratory activity, as rib muscle and diaphragm showed marked and early increases.

An unexpected finding was the high blood flow in brown fat at all stages, including deep hibernation. If tissue blood flow is an indication of tissue function, brown fat may act as a supply depot for readily mobilized lipids and possibly glycogen, which may then act as metabolic fuel during hibernation and the arousal
process. Most of the concepts of the metabolic fuel of arousal have been derived from respiratory quotient data. This quotient in hibernation is usually around 0.7 but may vary from 0.6 to 0.84, suggesting that a high proportion of fat is utilized in the oxidative metabolism. Mokrasch et al. (11) have reported that as the body temperature begins to rise in arousal the RQ is low, suggesting that lypolysis is accelerated. As warming continues RQ rises to 1.0 and then may fall again. However, in arousal with the increase in body temperatures, the decrease in the solubility of carbon dioxide, and marked increases in pulmonary ventilation, considerable caution must be used in interpretation of changes of the carbon dioxide to oxygen ratio. Such changes may reflect the immediate ventilatory exchange process. Most of the concepts of the metabolic fuel being utilized.

Lyman and Hastings (12) have shown that the total blood carbon dioxide content may be higher in hibernation than in awake hamsters and ground squirrels. The "breathing off" of this carbon dioxide may produce a higher ratio of heat conservation (3). Approximately 30-40% of the metabolic fuel of arousal has been derived from respiratory quotient data. This quotient in hibernation is usually around 0.7 but may vary from 0.6 to 0.84, suggesting that a high proportion of fat is utilized in the oxidative metabolism. Mokrasch et al. (11) have reported that as the body temperature begins to rise in arousal the RQ is low, suggesting that lypolysis is accelerated. As warming continues RQ rises to 1.0 and then may fall again. However, in arousal with the increase in body temperatures, the decrease in the solubility of carbon dioxide, and marked increases in pulmonary ventilation, considerable caution must be used in interpretation of changes of the carbon dioxide to oxygen ratio. Such changes may reflect the immediate ventilatory exchange process. Most of the concepts of the metabolic fuel being utilized. Lyman and Hastings (12) have shown that the total blood carbon dioxide content may be higher in hibernation than in awake hamsters and ground squirrels. The "breathing off" of this carbon dioxide may produce a higher ratio during arousal. Kaysar (13), by techniques of qualitative histochemistry and biochemical assay, has concluded that brown fat serves a storage function, being rich in both lipids and glycogen. Johansen (14) has completely reviewed the literature on the structure, occurrence, and suggested functions of brown fat. Further study is needed to elucidate the role of brown fat in arousal.

The centralization of blood flow and heat production in the thorax has been described as a useful mechanism of heat conservation (3). Approximately 30-40% of the cardiac output leaves the thoracic region at the arousal level of 100 beats/min. This would, in turn, remove some heat from the thorax and decrease the thoracic warming rate. However, it is conceivable that at all physiological levels a small amount of "maintenance" blood flow is necessary even to cooler and presumably inactive tissues.

Our concept of the circulatory changes in arousal now follows. During deep hibernation cardiac output is extremely low. Blood circulation is present in all tissues, but heart vessels, brown fat, and diaphragm receive a high portion of this reduced flow. As arousal commences esophageal temperature, heart rate, and cardiac output increase rapidly. The increase of blood flow is mostly in the heart muscle, brown fat, and respiratory muscles. The increased work of the heart and respiratory muscles and possibly increased shivering increase heat and metabolite production. These two factors may tend to dilate the local vasculature (15, 16). The heat produced in the tissues is carried back to the heart in venous blood and increases heart rate and output. Because of the local vasodilatation a large portion of the cardiac output increase would return to the active tissues and supply oxygen and metabolic fuels.

This process could best be described as a positive feedback process. As it continues, more and more tissues could be progressively brought into the system. Effective functional levels are reached before the circulation must be supplied to the entire animal. Nervous mediation must play a role in increasing peripheral anterior blood flow over posterior flow either by forcing activity in the anterior portions or by direct vascular control.

REFERENCES