Vascular response to changes in blood oxygen tension under various blood flow rates

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BACHOFEN, M., A. GAGE, AND H. BACHOFEN. Vascular response to changes in blood oxygen tension under various blood flow rates. Am. J. Physiol. 220(6): 1786 1792. 1971. The peripheral vascular response to Pao2 ranging from 25 to 2,500 mm Hg was systematically studied in the hindlimbs of dogs under constant-flow perfusion. With cross-circulation experiments using two dogs, a distinction between reflex-mediated response and local effects was possible. While reflex and local mechanisms, competing with each other, are responsible for the overall vascular reaction to hypoxia, the response to hyperoxia is shown to be a local effect only. The ventilation of oxygen at 3 and 4 Ata induced a 10-20% increase in perfusion pressure. Sympathectomized dogs showed a more pronounced response, i.e., 20-30% increase in perfusion pressure under comparable conditions, attributed to a hyperactivity of the denervated smooth muscle. Variations of the venous oxygen tensions between 40 and 700 mm Hg achieved by hyp-, normo-, and hyperperfusion of the hindlimbs during ventilation of pure oxygen at 3 Ata, did not influence the vasoconstrictive response. This result suggests a direct vasoconstrictor action of oxygen on the arterial and arteriolar wall, and rather excludes an indirect mechanism activated by changes in oxygen supply relative to tissue demand.

Bachofen et al. (1971) investigated the vascular response to changes in blood oxygen tension under various blood flow rates. They performed experiments in the hindlimbs of dogs and observed a local effect of hyperoxia on the peripheral vasculature. Sympathectomized dogs showed a more pronounced response, indicating a hyperactivity of the denervated smooth muscle. Variations in venous oxygen tensions did not influence the vasoconstrictive response, suggesting a direct action of oxygen on the arterial and arteriolar wall.

METHODS

Experiment A: Autoperfusion in Single Dogs

Mongrel dogs of either sex weighing 15-24 kg were anesthetized by intravenous injection of α-chloralose (approximately 80 mg/kg) or sodium pentobarbital (25-30 mg/kg). In order to maintain a constant alveolar ventilation during the whole course of the experiment, all dogs were intubated and mechanically ventilated. A jugular vein was cannulated for infusion purposes. Catheters were advanced into common carotid and right femoral arteries and left femoral vein through side branches to avoid strictures or ligations of major vessels. Arterial pressures from both locations, as well as venous pressure, were recorded with Hewlett-Packard pressure transducers (model 1280 C) connected to an eight-channel Sanborn recorder. A continuous recording of the perfusion pressure was necessary since sudden increases of vascular tone were observed during the course of some experiments. Such changes occurred unrelated to the momentary oxygen tensions and have to be attributed, most likely, to a spontaneous release of catecholamines. The test dog’s leg temperature was continuously monitored with a thin intravenous thermoprobe, and body temperature was kept constant with a heating pad beneath the animal’s body and infrared light.

The lower aorta was approached transperitoneally through a median laparotomy and cleared from side branches in an area of 2-3 cm above the bifurcation. After anticoagulation with heparin (3 mg/kg) and suitable control of blood flow with vascular clamps, the aorta was opened 2 cm above the bifurcation and a vinyl catheter loop (30 cm in length) was inserted to permit blood flow to the sacral branch of the aorta (i.e., the hindquarter) through the loop. The abdomen was then closed with the catheter loop protruding, making a connection to the roller pump possible within seconds. The blood pump was calibrated for different rotations per minute (rpm) over the desired blood flow range for the tubing used. A built-in Variac allowed us to change the blood flow rate during the experiment by a factor of 6 to 1. For most experiments, unless stated otherwise, the pump flow (200-280 ml/min) was adjusted to achieve a leg pressure similar to the systemic blood pressure.
Experiment B: Cross-Circulation Experiment With Two Dogs

Besides the above-mentioned autoperfusion in the single-dog experiment, there was a second type of perfusion carried out in which the hindlegs of the test dog were perfused from a donor animal (Fig. 1). Both dogs were prepared in essentially the same way as described, except for the vascular connections. In the donor dog, the catheter leading to the pump was introduced into the aorta proximal to the point of ligation (1 cm above the bifurcation), and in the test dog this catheter was inserted into the distal aorta. Similarly, a catheter was inserted into the proximal part of the abdominal vena cava and connected to the test dog by insertion into its distal vena cava to return venous blood to the donor. Arterial blood was pumped from the aorta of the donor animal to the bifurcation of the test animal. The venous return flow from the test dog to the vena cava of the donor dog was facilitated by the position of the donor dog, being 15-25 cm below the recipient dog. All blood gas measurements in the laboratory as well as in the hyperbaric chamber were obtained with an Instrumentation Laboratory pH and blood gas analyzer (model 113). The experiments under OHP were carried out in an 8 x 24 foot double-lock hyperbaric chamber at 3 or 4 Ata with the animals breathing a gas mixture equivalent to air at 1 Ata or 100% oxygen.

RESULTS

1) Effects of Breathing Low Oxygen Mixtures (Containing 14% and 10% O2, Respectively) Under Controlled Ventilation at 1 Ata

A) Single-dog experiments. The results of 20 experiments performed in eight different dogs are shown in Table 1. The systemic blood pressure measured in the carotid artery started to increase about 2 min after exposing the dogs to low oxygen mixtures; the pressure increase being more pronounced when the animals were exposed to the lower (10%) oxygen mixture. These findings are consistent, in general, with those of previous authors (13, 16).

On the other hand, the perfusion pressure measured in the femoral artery of the constant-flow perfused hindlimbs did not change significantly while ventilating the dogs with the 14% mixture. Subjected to 10% oxygen, however, a transient increase of perfusion pressure was observed in most experiments, starting simultaneously with the rise of systemic blood pressure but being less pronounced and resuming a steady state close to the preceding normoxic level after 4-7 min (Table 1). In four experiments, the perfusion pressure decreased to an even slightly lower value than during normoxia after the initial vasoconstriction.

Within about 1 min after reoxygenation (with air or oxygen), the systemic blood pressure dropped rapidly to its initial value; in a few cases of severe hypoxemia (Pao2, ranging between 25 and 35 mm Hg), it even showed a slight overshoot. Simultaneously, the hindlimbs responded with a vasodilatation: an abrupt, but transient, posthypoxic decrease of the perfusion pressure by 10-50% depending on the degree of hypoxemia, was followed by a gradual increase to its initial control level within about 1-2 min.

B) Cross-circulation experiments. Six experiments were performed in order to obtain an understanding of the peripheral vascular response described in the preceding paragraph, especially to distinguish between influences of central reflex mechanisms and local effect of hypoxemia. The results are given in Table 1. In addition, an example of typical results is shown in Fig. 2 A-C. In Fig. 2, A, B were consecutively obtained in the same experiment. First the test dog (Fig. 1) was ventilated with a 10% oxygen mixture, the donor dog received air and hence the hindlimbs of the test dog were perfused with normoxic blood. Figure 2A shows that this combination caused a clear vasoconstriction.

![Diagram of cross-circulation experiment](image)

**Fig. 1. Diagram of cross-circulation experiment. (BP = systemic blood pressure; PP = perfusion pressure.)**

### Table 1. Percent changes of systemic blood pressure and perfusion pressure in hindlimb due to different levels of hypoxia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent of Initial Pressure</th>
</tr>
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<tbody>
<tr>
<td>Pao2 = 0.14</td>
<td><strong>Single-dog experiment</strong></td>
</tr>
<tr>
<td>Pao2 = 31 ± 4 mm Hg</td>
<td>Increase in BP</td>
</tr>
<tr>
<td>Pao2 = 0.10</td>
<td>Transient increase in PP</td>
</tr>
<tr>
<td>Pao2 = 31 ± 5 mm Hg</td>
<td>Steady-state change of PP</td>
</tr>
<tr>
<td></td>
<td>Transient posthypoxic decrease of PP</td>
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<tr>
<td></td>
<td>Cross-circulation experiments</td>
</tr>
<tr>
<td></td>
<td>Change in PP during hypoxia of test dog</td>
</tr>
<tr>
<td></td>
<td>Change in PP during hypoxia of donor dog and hindlimb</td>
</tr>
</tbody>
</table>

Values are means ± sd. BP = systemic pressure; PP = hindlimb perfusion pressure. Steady-state change of PP (single-dog experiments) is to be considered as the resultant of both opposite PP changes obtained in the cross-circulation experiments described. FIO2 = oxygen fraction of inspired gas mixture; Pao2 = arterial oxygen tension. *Significant differences between related values (P < 0.02).
in the hindlimbs of the test dog, obviously due to a central reflex mechanism. The onset of the response started again after about 2 min and disappeared abruptly with a pronounced overshoot after the test dog was switched back to air breathing (posthypoxic reaction). In Fig 2B, the reaction of the hindlimb vessels to the inverse combination is shown: the test dog was ventilated with air, the donor dog with a low oxygen mixture, perfusing the hindlimbs with hypoxic blood. The local hypoxia in the legs induced a vasodilatation which subsided gradually upon reoxygenation. The summation of tracings 2A and 2B is compared with an original tracing obtained in a single-dog experiment (Fig. 2C).

9) Effects of Ventilation With 5.5% Oxygen and 100% Oxygen Alternately at 4 Ata

A) Single-dog experiments. Experimental results are given in Table 2. Additionally, the tracings of three experiments in which the vascular response could be repeatedly observed during at least four to six consecutive periods of normoxic and hyperoxic ventilation are displayed in Fig. 3. One to two minutes after the onset of hyperbaric oxygenation, the perfusion pressure started to rise gradually towards a 10–20% higher pressure level and reached a new steady state within 5–8 min. Experiments with a hyperbaric oxygen exposure up to 14 min confirmed this finding. Upon withdrawal of pure oxygen, the perfusion pressure returned to its control value.

The blood oxygen tension increased during hyperbaric oxygenation to 2,300–2,500 mm Hg in the arterial blood and to 370–1,200 mm Hg in the venous blood (from the left femoral vein). The corresponding control values during normoxia were found between 90 and 120 mm Hg (arterial) and between 30 and 60 mm Hg (venous). While the arterial Po₂ reached the new steady state within 5–6 min after
Table 2. Percent changes in perfusion pressure due to OHP

<table>
<thead>
<tr>
<th>Condition</th>
<th>Intact Animals</th>
<th>Sympathectomized Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 Ata O2</td>
<td>3 Ata O2</td>
</tr>
<tr>
<td>No of exps</td>
<td>Exposure lasting 8 min</td>
<td>Exposure lasting 10 min</td>
</tr>
<tr>
<td>PaO2, mm Hg</td>
<td>2,370±110</td>
<td>1,600±141</td>
</tr>
<tr>
<td>PV(O2), mm Hg</td>
<td>650±230</td>
<td>145±57</td>
</tr>
<tr>
<td>Change in PP (in % of normoxic value)</td>
<td>11.5±1.9</td>
<td>12.0±2.0</td>
</tr>
</tbody>
</table>

Mean values ± SD are given. PP = perfusion pressure; PaO2 = arterial oxygen tension; PV(O2) = regional venous oxygen tension.

After sympathectomy the vascular response to OHP is significantly increased as compared to intact animals tested at 2 different levels of OHP (P < 0.01).

Fig. 3. Peripheral vascular response to changes in inspired oxygen concentrations from 5.5% O2 (clear areas) to 100% O2 (shaded areas) at 1 Atm in 3 different dog experiments.

Changing the breathing mixture, at least 7-8 min were required for the venous blood.

In addition to the studies at 4 Ata, nine experiments have been carried out at 3 Ata in exactly the same way. Regardless of the considerably lower blood O2 tensions (ranging between 1,400 and 1,650 in the arterial and between 70 and 300 mm Hg in the venous blood), the percent increase of the perfusion pressure was found to be in the same range of between 10 and 20%.

B) Cross-circulation experiments. In order to rule out that the peripheral vasoconstriction observed is due, above all, to central reflex mechanisms (especially since the systemic blood pressure showed the tendency to decrease by a few millimeters Hg under hyperbaric oxygenation), identical cross circulation experiments as described in paragraph 1B were performed in the hyperbaric chamber at 4 Ata. The results are shown in Table 3. Moreover, two typical recordings of these experiments are displayed in Fig. 1. Exposure of the test dog only to hyperbaric oxygen (his hindquarters being perfused with normoxic blood) did not provoke any vasoconstriction in the separately perfused hindlimbs. However, an increase of the perfusion pressure similar to that found in the single-dog experiments was observed whenever the legs were perfused with oversaturated blood by ventilating the donor dog with pure oxygen, regardless of the oxygen concentration inspired by the test dog. Similarly, the perfusion pressure that returned to its orig-inal value after hyperoxigenation was discontinued in the legs, even if at the same time the test dog was ventilated with 100% oxygen. Obviously, the peripheral vasoconstriction due to hyperoxia has to be attributed exclusively to a direct local effect of high oxygen tension.

To confirm the results obtained thus far, a different approach was carried out to answer the same question of a possible reflex mechanism being involved in the vascular resistance change to hyperoxigenation.

C) Effect of hyperoxigenation after bilateral lumbar sympathectomy. Intermittent ventilation with 5.5 and 100% oxygen at 4 Ata was carried out in six dogs 5–7 days after a bilateral lumbar sympathectomy had been performed. Interruption of sympathetic transmission to the hindlimbs was considered to be complete if an intravenous injection of 0.1 mg epinephrine did not show any transient vasodilation in the constant-flow perfused legs. The pump and tubing system used for the constant-flow perfusion provided for a sufficient transport delay such that the reflex dilatation could clearly be seen before the onset of the vasoconstriction due to the local effect of the drug. This criterion seemed to be reliable since Folkow et al. (11) and Beck (2) have shown that the baroreceptor reflex is mediated by sympathetic vasoconstrictor fibers only. One experiment out of six had to be discarded because of incomplete sympathectomy.

The blood pressure in the sympathectomized dogs did not differ significantly from that observed in intact animals, a finding consistent with other authors (19), and, also, the blood flow necessary to achieve a perfusion pressure equal
to the systemic pressure was found to be in the same range of intact animals (between 200–280 ml/min). The results of these experiments are included in Table 2, and four uninterrupted recordings are displayed in Fig. 5. Although the flow rates and the blood oxygen tensions of the sympathectomized dogs were comparable to those of intact animals, the vasoconstriction due to high oxygen tensions was significantly more pronounced in the hindlimbs deprived of sympathetic innervation (Table 2). The increase in perfusion pressure was between 20 and 30% in all experiments. Additionally, it seemed that the response was also slightly faster, requiring only 4–5 min (compared to 5–8 min) to reach the new steady state upon changing the gas mixture. Besides this difference, the results are further evidence for the local effect of hyperbaric oxygen.

3) Effect of Hypo- and Hyperperfusion on Response to Hyperoxygenation at 3 Ata

After experiments at 3 Ata had shown an almost identical response to hyperbaric oxygenation as those carried out at 4 Ata, the following studies were performed at 3 Ata which allowed a longer compression time (bottom time) without the inconvenience of a long decompression.

In the first group, experiments were started as usual, but the inspired gas was changed only twice, from 7% oxygen to 100% oxygen and back to 7% oxygen. Thereafter the blood flow to the hindlimbs was reduced from normal (200–280 ml/min) to 90 ml/min. As soon as a steady state was reached at the lower perfusion pressure, the same ventilation cycles were repeated several times.

In the second group of experiments, the flow was increased from normal to 450–500 ml/min, and the vascular response to high oxygen tensions was tested under this condition in the same way. Figure 6 and Table 4 show the results of these studies. The higher the perfusion pressure, the higher is the absolute pressure increase due to hyperbaric oxygenation. Expressed in percent of perfusion pressure, however, the pressure increase seemed to be nearly constant, regardless of whether the hindlimbs were hypoperfused, normoperfused, or hyperperfused. (The differences are insignificant, $P > 0.05$.) Since the preparations examined responded more or less as a passive vascular bed (a peculiarity which may be attributed to the length of the experiments (22)), the vascular resistance did not change considerably by increasing or decreasing the perfusion rate. Thus, the resistance changes due to OHP at different flow rates are well comparable, indicating that the vascular response to

![Fig. 5. Peripheral vascular response to changes in inspired oxygen concentrations from 5.5% O₂ (clear areas) to 100% O₂ (shaded areas) at 4 Ata in 4 different sympathectomized dogs.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2982573/)

![Fig. 6. Vascular response to 100% oxygen at 3 Ata under hypo-, normo-, and hyperperfusion. Each upper tracing (dotted line) represents absolute change in perfusion pressure in a typical experiment. In lower tracing, average of percent changes in perfusion pressure is indicated by solid line; range of percent change is illustrated by areas with diagonal bars. Corresponding changes in PaO₂ are shown by clear columns, changes in Pvo₂ by black columns. (Note that P₂ scale is logarithmic.)](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2982573/)

### TABLE 4. Arterial and regional venous oxygen tensions and relative increase in perfusion pressure due to oxygen under high pressure in hypo-, normo-, and hyperperfused hindlimbs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hypoperfusion, ~90 ml/min</th>
<th>Normoperfusion, ~225 ml/min</th>
<th>Hyperperfusion, ~450 ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPaO₂, mm Hg</td>
<td>92</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>PvO₂, mm Hg</td>
<td>24</td>
<td>35</td>
<td>54</td>
</tr>
<tr>
<td>PPaO₂, mm Hg</td>
<td>1,660</td>
<td>1,630</td>
<td>1,700</td>
</tr>
<tr>
<td>Pvo₂, mm Hg</td>
<td>44</td>
<td>70</td>
<td>640</td>
</tr>
<tr>
<td>% Change in perfusion pressure*</td>
<td>$+18.5 \pm 2.7$</td>
<td>$+17.0 \pm 4.3$</td>
<td>$+18.0 \pm 6.0$</td>
</tr>
</tbody>
</table>

Average values of 3 experiments are given. The same data are illustrated in Fig. 6. PPaO₂ = arterial oxygen tension; Pvo₂ = venous oxygen tension; PpaO₂ = inspired oxygen tension. *Means $\pm$ se.
OHP was about identical under all three experimental conditions. Therefore, the vascular response to high oxygen tension does not appear to be modified by the oxygen tension of the regional venous blood and its increase following hyperoxegenation. After changing the inspired oxygen concentration from 7 to 100%, the PV_{O2} in the leg increased from about 25 to 45 mm Hg at low flow, from 35 to 70 mm Hg at normal flow, and from 50 to 600 mm Hg at high flow, whereas the arterial oxygen tensions were practically identical for all flow rates.

**DISCUSSION**

Since the hindlimbs were artificially perfused, the relevance of the perfusion technique employed to the vascular response has to be discussed. The main advantage of constant-flow perfusion is the strict proportionality between the rise in driving pressure and the active tension of the vessel wall (4). Folkow (10) maintained that constant flow perfusion causes a decrease in vascular tone and reactivity. Based on systematic experiments, Davis and Hammond (8) reached a similar conclusion, but, as they pointed out, the diminished vascular response during constant-flow perfusion, as compared to constant-pressure perfusion, had to be attributed largely to the extremely high perfusion pressures applied. In contrast to that, the perfusion pressures were kept close to the normal systemic blood pressure in the present study. Jones and Berne (15) found that initiation of pump perfusion at the same flow level as existed under constant pressure produced a slight fall in perfusion pressure. But in contrast to Folkow's observation, they were able to show the same vascular response in the isolated muscle, regardless of whether they increased or decreased either the blood flow or the perfusion pressure. Examples of normal vascular reactivity under pump perfusion have also been shown in the intestinal vascular bed (9). Finally, Costin and Skinner (6) described, in normally perfused skeletal muscles, a vascular response to hypoxemia which is almost identical to the one we observed during constant-flow perfusion. These considerations suggest that the method applied has probably a minor influence on the results.

In general, the hypoxia experiments confirm the results of other authors (6) (although the method applied was substantially different), and hence need not to be discussed in detail. The cross-circulation experiments demonstrate clearly that the peripheral vascular response to incomplete arterialization of blood in the lung is a complex one (Fig. 2, A–C). According to our results, the vascular resistance changes observed during hypoxemia and on reoxygenation, such as described by Robert and Haab (20), are the resultant of two opposite responses: a vasoconstriction due to a chemoreceptor reflex which can be modified by the ventilation (1, 7, 17), and a vasodilation due to the local effect of hypoxemia on the vessel wall (14, 22). Consequently, the posthypoxic vasodilatation is due to an overshooting vascular relaxation after termination of the chemoreceptor induced vasoconstriction. Essentially the same results as shown in Fig. 2, A–C, were indirectly obtained by Costin and Skinner (6), who abolished the reflex mechanism either by denervation of skeletal muscles or by drug-induced nerve blockade.

In contrast to that, the vasoconstriction due to hypoxemia seems to be unaffected by neural regulation. Neither a reflex mechanism via baroreceptor (OHP generally caused a slight drop in systemic blood pressure), nor a possible stimulation of medullary vasomotor arcs by hypoxemia appears to detectably modify this response. Best evidence for this conclusion is given by the cross-circulation experiments. Subjecting the test dog (Fig. 1) to hyperbaric oxygen resulted in no change in the vasomotor tone of its normoxic blood-perfused hindlimbs (Table 3). But vice versa, vasoconstriction occurred without exception and to the same extent in the hindlegs exposed to high oxygen tensions regardless of whether the test dog was ventilated with a normoxic or hyperoxic gas mixture (Fig. 4).

Although the cross-circulation experiments give evidence that the vascular response to hyperoxia is not mediated by the autonomic nervous system, they are not conclusive as to whether the vasoconstriction is caused by circulating humoral mediators increased by OHP, or by a direct vascular stimulation. However, experiments performed by Walker (23) seem to support the second alternative. This author found a clear vasoconstriction due to OHP in isolated hindlimbs perfused with Tyrode dextran solution. But, since the experimental conditions of the present study and those of Walker (23) are quite different, a quantitative comparison is impossible. Consequently, a humoral contribution to the direct action of hyperoxia cannot be completely denied in the intact animal.

In addition to the cross-circulation experiments, studies on sympathectomized dogs were carried out to further rule out a reflex mechanism. Unexpectedly at first, the response to hyperoxegenation turned out to be significantly more pronounced than in intact animals. Initially, this observation might appear as a contradiction to the findings and conclusions above, i.e., that hyperoxic vasoconstriction is not the result of neural activity. However, the enhanced vasoconstriction is best explained by the "law of denervation" which was formulated several decades ago by Cannon (5). Accordingly, denervated smooth muscles react with increased irritability to chemical stimuli. It has been shown experimentally, for example, that vasoconstriction due to circulating catecholamines is more severe in denervated organs (12). There is no direct experimental evidence that the "law of denervation" is applicable to high oxygen tensions as stimulus; and one might well argue that the enhanced effect of OHP after sympathectomy is pointing to an increased release of a circulating vasoconstrictor substance.

Oxygen under increased pressure always induced a local vasoconstriction, but the resulting flow reduction (inversely proportional to the resistance increase) was never severe enough as to counteract entirely the benefit from the increased oxygen-carrying capacity in the arterial blood, this result confirms the observations of Bergofsky and Bertun (3) and Whalen et al. (24).

The purpose of the third part of this study, pertaining to variations of perfusion rates, was to further investigate the mechanism of hyperoxic vasoconstriction. After excluding a neural regulation (by the cross-circulation experiments described) and a predominant influence of circulating vasoactive compounds (23), there are still three possible
mechanisms left. First, a direct reaction of vessel smooth muscles to high arterial oxygen tension; second, tissue hypoxia causing a release of vasoactive compounds that in turn diffuse to the arterioles and induce vasconstriction; and third, a combined action of both these mechanisms. Stainsby and Otis (21), and Bergofsky and Bertun (3) have shown that the oxygen consumption of resting muscle is not altered by changes in blood flow or blood oxygen tensions, except during severe hypoxia. Hence, by increasing the perfusion rate the ratio between oxygen supply and demand and also the regional venous 

Paz increases, although not altered by changes in blood flow or blood oxygen tension. Hyperbaric oxygen is upstream from the capillary bed.

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