The effect of carbon monoxide on respiration

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Summary. In this review the effects of carbon monoxide on tissular oxygenation, at doses which are compatible with life, are considered. In a first section the relative CO-O_2 affinity (M*) of various O_2 carrying proteins is compared; M* is about 220 for hemoglobin, 20–25 for myoglobin and close to unity for cytochrome oxidases. Thus most of the acute CO toxicity should not be considered as due to malfunction of the intracellular respiratory chain. In addition the differences in M* are caused more by the changes in O_2 affinity than by those in CO affinity. The second section deals with the changes in the O_2 equilibrium curve (OEC) induced by the presence of HbCO in blood, i.e. the hyperbolization of this curve due to the progressive loss of allostery due to the preferential binding of CO to Hb. The functional importance of this phenomenon lies in the fact that the lower part of the OEC is shifted to the left, whereas the upper part is shifted to the right to an extent which depends upon the amount of HbCO. Thus the effects of the so-called CO anemia are considered to be due both to the reduction of functional Hb and to the reduced partial pressure in the hypoxic range of the OEC. The third section presents recent data concerning the effect of HbCO on the $\dot{V}_{O_2}$max of the isolated gastrocnemius preparation. The results were obtained in hypoxia under conditions where perfusion and arterial O_2 content, i.e. O_2 delivery, were the same with and without 30% HbCO. The salient finding is a 26% reduction of $\dot{V}_{O_2}$max under conditions of CO anemia as compared to hypoxia alone. Interestingly, the P_{O_2} of the venous effluent of the muscle is found to be the same in both cases which leads to the interpretation that it is not the reduction of the mean capillary P_{O_2} but rather a decrease of the blood-to-mitochondria O_2 conductance which causes the fall in $\dot{V}_{O_2}$max.

Key words. Carbon monoxide; hypoxia; CO-O_2 competition; carboxyhemoglobinemia; tissue O_2 supply; CO poisoning.

As early as the 17th century[21], carbon monoxide (CO) was recognized as a potentially life-threatening toxic gas. Since the end of the last century this toxicity has been thought to be due to the very high affinity of CO for oxygen carrier proteins, mainly hemoglobin. Normally, the blood CO concentration does not exceed 1–2% of the blood CO carrying capacity, and consequently it does not interfere with blood O_2 transport. However, when CO is taken up by the lungs from inspired air, CO-bound hemoglobin, HbCO, can easily increase to levels that impair O_2 transport in the blood for two reasons: firstly, the concentration of the functional hemoglobin is decreased – an effect often called CO anemia; secondly the O_2 affinity of the functional, not CO-bound, hemoglobin is increased. CO is exchanged in the tissues as easily as in the lungs, so that it can bind to extravascular proteins such as myoglobin and cytochromes[3]. Therefore it can also interfere with the transport and respiratory functions of those proteins.

CO affinity for O_2 carrier proteins

Table 1 gives accepted values for the partial pressures of CO and of O_2 which saturate 50% of the binding capacity of three hemoproteins; hemoglobin, myoglobin and cytochrome oxidase aa_3. These partial pressures, known as P_{50}, are the reciprocals of the affinities of the corresponding gases at half saturation; thus the relative affinity of CO as compared to that of O_2 is given by the ratio $P_{50}O_2/P_{50}CO$, called M*. CO binds chemically, like O_2, to the divalent iron atom of the heme in all three hemoproteins, but the affinities differ very much depending on the hemoprotein. For hemoglobin, CO has an affinity 200–250 times larger than O_2, for myoglobin 20–25 times, whereas for cytochrome aa_3 the relative affinity is about unity or even less.

The $P_{50}CO$ values of the three hemoproteins are of the same order of magnitude, which suggests that the structural constraints for the CO binding are similar for the three proteins. Thus, the larger differences in their CO-O_2 relative affinities result mainly from the large differences exhibited by their $P_{50}O_2$ values, that for Hb being one and two orders of magnitude greater than for myoglobin and for cytochrome aa_3, respectively.

CO affinity for whole blood and CO-O_2 relative affinity

Haldane has defined a CO-O_2 relative affinity factor which he called M for the conditions where blood is equilibrated simultaneously with O_2 and CO. This factor may be thought of as the ratio of the apparent equilibrium constants for CO, L_{CO}, and for O_2, K_{O_2}.

$$L_{CO} = [HbCO]/[Hb] \cdot [CO]; \; K_{O_2} = [HbO_2]/[Hb] \cdot [O_2]$$
$$\text{then } L_{CO}/K_{O_2} = HbCO \cdot P_{O_2}/HbO_2 \cdot P_{CO} = M$$

Haldane found that, as long as P_{O_2} and P_{CO} were large enough to fully saturate hemoglobin, the value of M was independent of the relative amounts of HbCO and HbO_2. This is often called Haldane's 'first law'. Since Haldane believed that the CO and O_2 equilibrium curves, COEC and OEC, were isomorphous he also proposed that his first law be applicable when P_{CO} and P_{O_2} do not

Table 1. Values of $P_{50}O_2$, $P_{50}CO$ and of relative CO-O_2 relative affinity, M* for hemoglobin, myoglobin and cytochromes aa_3 in various mammalian species

Hemoproteins	$P_{50}O_2$ (Torr)	$P_{50}CO$ (Torr)	M* ($P_{50}O_2/P_{50}CO$)
Human hemoglobin A (blood in vitro)	26.7	0.125	215
Myoglobin	1.3–5.3	0.05–0.2	25–20
Cytochromes aa_3	0.5–0.6	≈ 1	≈ 0.5

fully saturate Hb. This has been called Haldane's 'second law'. Since it is now established that the CO-O_2 relative affinity, M*, is lower at lower saturation, Haldane's M is also expected to decrease at low Hb saturation and even more so the larger the HbCO. Whereas Haldane's first law has been amply verified, his second law is considered to be of limited value [23], although measurements of M in desaturation are scarce. Joels and Pugh [19] also observed that, at full saturation, M and M* were not identical, a disparity which could be explained by the fact that the chemical definition of M involves more equilibrium constants than M*.

Effects of CO on position and shape of the oxygen equilibrium curve

In 1912, Douglas et al. [7] and Haldane [11] discovered the fact that HbCO in blood induces a hyperbolization of the OEC. In doing so they elucidated one of the most important features of carbon monoxide toxicity: in addition to reducing the amount of functional hemoglobin available for O_2, CO augments the O_2 affinity of the remaining functional hemoglobin, and consequently reduces the blood O_2 partial pressure and thus the driving force for O_2 diffusion to peripheral tissues. They have very clearly shown that tissue oxygenation is reduced more by a decrease in functional Hb through CO anemia, than by a reduction of the hemoglobin concentration by an equal percentage, i.e. simple anemia.

Figure 1 illustrates the hyperbolizing effects of CO, redrawn mostly after Zwart et al. [32] but in full accordance with recent data of Okada et al. [23] and of Hlastala et al. [13]. Panel A represents the O_2 saturation versus P_{O_2} for different concentrations of HbCO: 0, 20, 40 and 60% corresponding to the curves 1, 2, 3, and 4 respectively. It is seen that the curves for HbCO-containing blood are shifted progressively to the left. Panel B represents the effect of the same HbCO concentrations on the O_2 content versus P_{O_2} relationships. These curves, relevant for the analysis of gas exchange, show a simultaneous decrease in O_2 capacity and OEC hyperbolization; each curve intersects the normal curve at a progressively lower P_{O_2} as HbCO increases. On such curves the term 'left shift' may be misleading since it applies only to the part below the point of intersection; above this point, in contrast, the curves are shifted to the right. Because of this, the effects of the left-shifted OEC on tissue oxygenation are most pronounced in hypoxia.

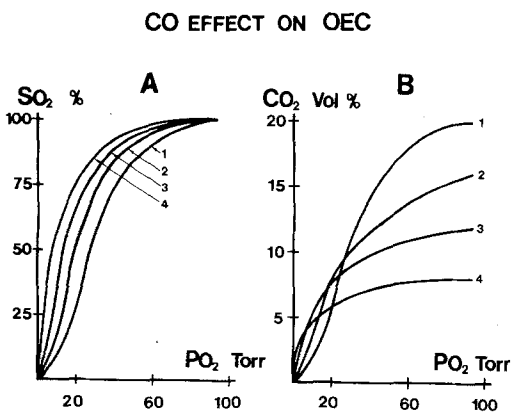

Figure 1. Effect of various HbCO concentrations on O_2 equilibrium curve of human blood. The curves 1, 2, 3 and 4 correspond to HbCO concentrations of 0, 20, 40 and 60% respectively. Panel A: O_2 saturation, Panel B: O_2 content, both versus P_{O_2}.

CO effect on O_2 transport from capillaries to mitochondria

To reach the site of its consumption, O_2 must first be detached from hemoglobin and move out of the erythrocytes to proceed through various tissue components, such as the capillary wall, the interstitial space and finally the cell membrane to its final destination, the mitochondria. Hence, it must overcome many resistances in series. By analogy to what has been proposed for the lung [8], these resistances can be considered as two: a blood resistance, including a chemical component due to off-rate kinetics of deoxygenation, and a tissular resistance. Overcoming of the second resistance may be facilitated in contractile tissues by the carrier-assisted transport of myoglobin [6, 30]. Thus, after the blood resistance, two resistances in parallel, corresponding to simple and facilitated diffusion respectively, must be considered. Carbon monoxide may affect the value of the overall conductance for O_2 of the blood to tissue path by way of its competition with O_2 on hemoglobin and myoglobin.

The overall blood-to-tissue O_2 conductance, D_{tiss}, may be defined from the general transfer equation:

$$\dot{V}_{O_2} = D_{tiss}(Pc_{O_2} - P_{tiss}O_2)$$

where D_{tiss}, the apparent tissue diffusing capacity, is a combined parameter comprising the above-mentioned resistances, Pc_{O_2}, the mean capillary P_{O_2}, and $P_{tiss}O_2$, the tissue P_{O_2} in the immediate vicinity of the mitochondria. In real organs, the $Pc_{O_2} - P_{tiss}O_2$ difference and consequently the value of D_{tiss} are not defined by diffusion only, but also by such factors as uneven distribution of blood flow, and shunts, which are not affected by CO. However when $\dot{V}_{O_2}$ is large the diffusion limitation may become predominant and the adverse effects of CO more evident.

It has been shown in smokers that $\dot{V}_{O_2}max$ is reduced by about 1% per % HbCO [12]; isolated muscles working in normoxia but with 50% HbCO have an oxygen con-

sumption 16% lower than in hypoxia[20], whereas the same preparation working at maximal aerobic capacity exhibits a 26% reduction in $\dot{V}_{O_2}$max when perfused with hypoxemic blood containing 30% HbCO as compared to hypoxia with no CO[10]. At rest, however, CO does not induce any change in $\dot{V}_{O_2}$ in normoxic man[18] nor in hypoxic dog[28]. Recent studies performed on myocardial cells in culture showed that the growth rate and the beating rate of muscle cells is not affected by addition of up to 20% CO to the incubation gas; in contrast the growth rate of non-muscle cells was paradoxically reduced in the presence of CO, even when the O_2 concentration was maintained at the normal level[22]. These observations suggest an adaptation of the metabolism of the cardiac cells in culture. In vivo, however, the myocardial O_2 consumption has been shown to undergo a small decrease under the influence of CO[1].

Quantitative analysis of the different effects of CO on $\dot{V}_{O_2}$max is difficult because of their simultaneous occurrence. Firstly, the left shift of the OEC must certainly play a role in diminishing the driving forces for O_2 diffusion through tissues which contain no myoglobin. However, no proportionality is expected between this effect and HbCO concentration since the left shift does not increase linearly with CO concentration (fig. 1, panel B). Secondly, the off-rate reaction kinetics of oxygen are expected to be slower in the presence of CO, and the role of this factor has been suspected to be important[25]. The O_2 off-rate is known to depend upon the absolute concentration of HbO_2 and upon capillary P_{O_2}, both of which are reduced in the presence of CO. Thirdly, the well-established carrier function of myoglobin is complex because this function depends on its degree of oxygenation. When myoglobin is completely blocked by H_2O_2[5] or by CO[30], 30–50% of the O_2 transfer is suppressed. Myoglobin binds CO with a relative CO-O_2 affinity of 20–25 (table 1) and has been shown to be only 20–30% O_2-saturated in maximally working muscles, where it acts as an O_2 redistributor and thereby as a P_{O_2} buffer[17]. Therefore, relatively large reductions of $\dot{V}_{O_2}$max due to the presence of MbCO must be predicted.

Effect of carboxyhemoglobinemia on $\dot{V}_{O_2}$ max of isolated muscle

The study referred to here[10] was designed to investigate the hypothesis that diffusion limitation is an important determinant of $\dot{V}_{O_2}$max in the pump-perfused isolated gastrocnemius preparation. To this end, this preparation was studied under conditions of maximal work in normoxia, hypoxia and hypoxia plus carboxyhemia. In theory, if other factors remain the same, a left-shifted OEC should increase the rate at which the capillary to tissue P_{O_2} declines as O_2 is removed by the working tissues, thereby reducing the capillary-to-tissue P_{O_2} driving gradient along the capillary length. It was postulated that if indeed this driving gradient is the important determinant of total O_2 flux into the tissue, the faster fall in P_{O_2} along the capillary length with the left-shifted OEC should reduce the $\dot{V}_{O_2}$max. Caution was taken to maintain blood CO partial pressure (P_{CO}) very low and to keep the exposure of the muscle to the blood containing CO very short, to minimize CO movement into the tissue. In hypoxia, carboxyhemoglobinemia offers the possibility of delivering to a muscle blood having the same P_{O_2} (Pa_{O_2}), O_2 content (Ca_{O_2}) and total [Hb], but a differently shaped OEC. Accordingly, the study compares muscle $\dot{V}_{O_2}$max under two conditions: 1) hypoxemia alone, i.e. hypoxic hypoxia (HH) and 2) hypoxemia with carbon monoxide (CMH). The choice of 30% HbCO, based on pilot measurements on dog blood, allowed equal Ca_{O_2} and Pa_{O_2} under both conditions at a Pa_{O_2} of 30 Torr. Because the blood flow ($\dot{Q}$) to the muscle was kept the same during both conditions, the O_2 delivery ($\dot{Q} \times Ca_{O_2}$) to the muscle was the same in HH and CMH.

The major data are presented in table 2. It is seen that on going from normoxic to HH conditions the O_2 delivery ($\dot{Q} \cdot Ca_{O_2}$) decreases by 32% and that concomitantly $\dot{V}_{O_2}$max decreases by 29%. Such a result is in full accordance with the classical observation of Stainsby and Otis[29] showing that below a critical value $\dot{V}_{O_2}$ falls linearly as O_2 delivery falls. Figure 2 is a plot of $\dot{V}_{O_2}$max against

Table 2. Major gas exchange data obtained on maximally working, pump-perfused isolated gastrocnemius, under normoxia, hypoxic hypoxia and hypoxia associated with 30% carboxyhemoglobinemia (mean values ± SEM)

		Hypoxia without CO (HH)	Hypoxia with CO (CMH)	Normoxia without CO
O_2 delivery	($ml \cdot min^{-1}$)	12.1 ± 0.7	12.2 ± 0.7	17.9 ± 1.8
Pa_{O_2}	(mm Hg)	30 ± 1	31 ± 1	76.0 ± 2.8
$\dot{V}_{O_2}$	($ml \cdot min^{-1}$)	8.8 ± 0.6	6.5 ± 0.4	12.4 ± 0.7
Pv_{O_2}	(mm Hg)	16 ± 1	16 ± 1	24.5 ± 1.4

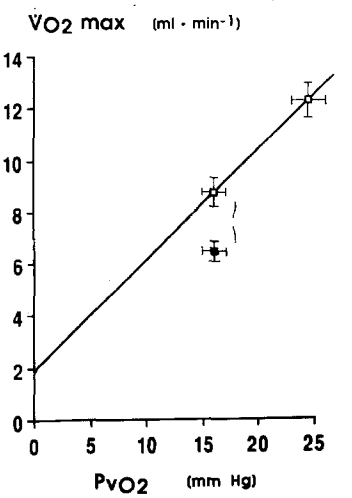

Figure 2. Isolated muscle $\dot{V}_{O_2}$max as a function of P_{O_2} of the venous effluent blood from the muscle. Open squares: normoxia and hypoxic hypoxia; filled square: hypoxia associated with 30% carboxyhemoglobinemia.

Pv_{O_2}, the P_{O_2} of the muscle effluent blood. It is apparent that the line passing through the normoxic and the HH value extrapolates to $\dot{V}_{O_2}max$ value which is not different from zero for $Pv_{O_2} = 0$. This observation agrees very well with those of Wagner's group in San Diego in man and in dogs [14, 15, 22]; this is interpreted as being strong evidence in favor of the diffusion limitation of $\dot{V}_{O_2}max$.

As far as the effect of carboxyhemoglobinemia under isodelivery conditions is concerned, the principal observation was that $\dot{V}_{O_2}max$ was 26% less during CMH, the difference between the two conditions being highly significant ($p < 0.01$). The O_2 extraction ratio $(Ca_{O_2} \cdot Cv_{O_2})/Ca_{O_2}$ was therefore also 26% less in CMH. The P_{O_2} of the muscle effluent venous blood, Pv_{O_2}, was the same, 16 ± 1 Torr in both conditions, indicating a large difference in the venous O_2 contents (Cv_{O_2}). It must be noted that repeated bouts of work under HH and CMH conditions showed that the effect of HbCO blood was quickly reversible. In addition, the difference in $\dot{V}_{O_2}max$ between HH and CMH did not depend on whether the CMH treatment was first or second in the order of matched treatment pairs. In this study, the left-shifted ODC during HbCO was expected to produce a lower mean capillary P_{O_2}, which for the same Pa_{O_2}, O_2 delivery, and tissue O_2 diffusing capacity, would result in a lower $\dot{V}_{O_2}max$. However, it was found that the P_{O_2} of the muscle venous effluent blood was the same in both HH and CMH. Had the OEC been linear in both conditions, this unexpected finding would show that the mean capillary P_{O_2} would also be identical in both cases. Even taken into account the small curvatures of the OECs over the range covered by the O_2 extraction, the identity of the Pv_{O_2} values indicate that the mean capillary P_{O_2} must have been similar in both HH and CMH conditions. Therefore the fall in $\dot{V}_{O_2}max$ during CMH cannot be considered to be due to a change in the capillary driving pressure for O_2, as estimated by mean capillary P_{O_2}; it must be concluded that the blood-to-tissue O_2 diffusive conductance was diminished during CMH. Computations of tissue D_{O_2} using a Bohr integration procedure show that the apparent D_{O_2} decreases by about the same amount as $\dot{V}_{O_2}max$ during CMH. Thus these results indicate that perfusion with HbCO blood impairs the ease with which O_2 can be transferred from the blood capillary to the mitochondria.

CO myoglobin affinity is some 30 times larger than that for O_2 [4]. Taking into account a myoglobin P_{50} for CO of 0.16 Torr and a myoglobin saturation of 30% in maximally working muscles [18] one can estimate that for a PCO of 0.07 Torr, myoglobin would be only 25% saturated with CO at equilibrium. Thus it seems impossible to attribute the whole decrease of O_2 conductance to a blockade of the myoglobin-facilitated O_2 transfer.

It is known that in the lung the reaction kinetics can limit the rate of pulmonary O_2 uptake [26]. Thus, one possibility that could explain our data is that the kinetics of O_2 release from the erythrocytes is sufficiently slow to impose a 'diffusion' resistance to O_2 transport, as was suggested by Rose and Goresky [25] in the coronary circulation. Although the initial off-rate reaction depends only on the concentration of HbO_2 [16], which is the same in HH and CMH conditions, it can be suspected that the presence of HbCO could modify the time-course of deoxygenation [27]. Such a role for off-loading kinetics is consistent with the predictions of Gutierrez [9].

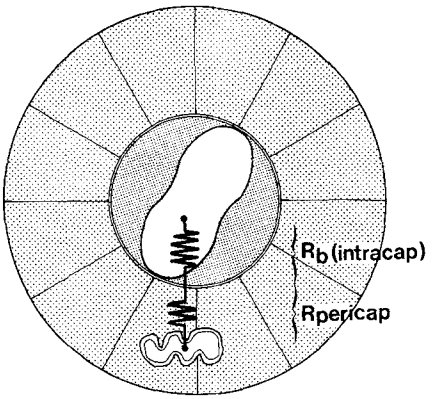

Figure 3. Hypothetical model of a centrally perfused (pseudo-kroghian) muscle fiber, showing two resistances in series on the hemoglobin to mitochondria O_2 path. The first resistance contains the off reaction kinetics of HbO_2, the second considers both simple diffusion and parallel myoglobin mediated facilitated diffusion.

In conclusion, the data presented in table 2 not only demonstrate that O_2 delivery cannot be regarded as the sole determinant of $\dot{V}_{O_2}max$ in the isolated gastrocnemius preparation, but they also confirm that two resistances for O_2 must be taken into consideration (fig. 3), the first being intracapillary and dependent upon the off-rate kinetics of HbO_2, and the second being pericapillary and dependent upon the carrier function of myoglobin. The presence of carbon monoxide in blood is likely to influence both of these resistances.

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1 Adams, J. D., Erickson, H. H., and Stone, H. L., Myocardial metabolism during exposure to carbon monoxide in the conscious dog. J. appl. Physiol. *34* (1973) 238–242.
2 Ayres, S. M., Giannelli, S. Jr, and Meuller, H., Myocardial and systemic response to carboxyhemoglobin. Ann. N.Y. Acad. Sci. *174* (1970) 268–293.
3 Coburn, R. F., The carbon monoxide body stores. Ann. N.Y. Acad. Sci. *174* (1970) 11–22.
4 Coburn, R. F., and Mayers, L. B., Myoglobin O_2 determined from measurements of carboxymyoglobin in skeletal muscle. Am. J. Physiol. *220* (1971) 66–74.
5 Cole, R. P., Skeletal muscle function in hypoxia: effect of alteration of intracellular myoglobin. Respir. Physiol. *53* (1983) 1–14.
6 De Koning, J., Hoofd, L. J. C., and Kreuzer, F., Oxygen transport and the function of myoglobin. Theoretical model and experiments in chicken gizzard smooth muscle. Pflügers Arch. *389* (1981) 211–217.
7 Douglas, C. G., Haldane, J. S., and Haldane, J. B. S., The laws of combination of haemoglobin with carbon monoxide and oxygen. J. Physiol., Lond. *44* (1912) 275–304.
8 Forster, R. E., Roughton, F. J. W., Cander, L., Briscoe, W. A., and Kreuzer, F., Apparent pulmonary diffusing capacity for CO at varying alveolar O_2 tensions. J. appl. Physiol. *II* (1957) 277.

9 Gutierrez, G., The rate of oxygen release and its effect on capillary O_2 tension: a mathematical analysis. Respir. Physiol. *63* (1986) 79–96.
10 Haab, P. E., Hogan, M. C., Bedout, D. E., Gray, A., Wagner, P. D., and West, J. B., Limitation of O_2 uptake in working muscle due to the presence of carbon monoxide in blood. Faseb J. *2* (1988) A760.
11 Haldane, J. B. S., The dissociation of oxyhemoglobin in human blood during partial CO-poisoning. J. Physiol., Lond. *45* (1912) XXII.
12 Hirsch, G. L., Sue, D. Y., Wasserman, K., Robinson, T. E., and Hansen, J. E., Immediate effects of cigarette smoking on cardio-respiratory responses to exercise. J. appl. Physiol. *58* (1985) 1975–1981.
13 Hlastala, M. P., McKenna, H. P., Franada, R. L., and Detter, J. C., Influence of carbon monoxide on hemoglobin-oxygen binding. J. appl. Physiol. *41* (1976) 893–899.
14 Hogan, M. C., Roca, J., Wagner, P. D., and West, J. B., Limitation of maximal O_2 uptake and performance by acute hypoxia in dog muscle in situ. J. appl. Physiol. *65* (1988) 815–821.
15 Hogan, M. C., Roca, J., Wagner, P. D., and West, J. B., Dissociation of maximal O_2 uptake from O_2 delivery in canine gastrocnemius in situ. J. appl. Physiol. *66* (1989) 1219–1226.
16 Holland, R. A. B., Shibata, H., Scheid, P., and Piiper, J., Kinetics of O_2 uptake and release by red cells in stopped-flow apparatus: effects of unstirred layer. Respir. Physiol. *59* (1985) 71–95.
17 Honig, C. R., Frierson, J. L., and Gayeski, T. E. J., Anatomical determinants of O_2 flux density at coronory capillaries. Am. J. Physiol. *256* (1989) H375–H382.
18 Honig, C. R., Gayeski, E. J., Federspiel, W., Clark, A. Jr, and Clark, P., Muscle O_2 gradients from hemoglobin to cytochrome: new concepts, new complexities. Adv. exp. Med. Biol. *169* (1984) 23–38.
19 Joels, N., and Pugh, L. G. C. E., The carbon monoxide dissociation curve of human blood. J. Physiol., Lond. *142* (1958) 63–77.
20 King, C. E., Dodd, S. L., and Cain, S. M., O_2 delivery to contracting muscle during hypoxic or CO hypoxia. J. appl. Physiol. *63* (1987) 726–732.
21 Moray, R., A relation of persons killed with subterraneous damps. Phil. Trans. R. Soc., Lond. *1* (1665) 44–45.
22 Nag, A. C., Chen, K. C., and Cheng, M., Effects of carbon monoxide on cardiac muscle cells in culture. Am. J. Physiol. *255* (1988) C291–C296.
23 Okada, Y., Tyuma, I., Ueda, Y., and Sugimoto, T., Effect of carbon monoxide on equilibrium between oxygen and hemoglobin. Am. J. Physiol. *230* (1976) 471–475.
24 Roca, J., Hogan, M. C., Story, D., Bebout, D. E., Haab, P., Gonzalez, R., Ueno, O., and Wagner, P. D., Evidence for tissue diffusion limitation of $\dot{V}_{O_{2max}}$ in normal humans. J. appl. Physiol. *67* (1989) 291–299.
25 Rose, C. P., and Goresky, C. A., Limitations of tracer oxygen uptake in the canine coronary circulation. Circ. Res. *56* (1985) 57–70.
26 Roughton, F. J. W., and Forster, R. E., Relative importance of diffusion and chemical reaction rates in determining rate of exchange of gases in the human lung, with special reference to true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J. appl. Physiol. *11* (1957) 290–302.
27 Roughton, F. J. W., Forster, R. E., and Candor, L., Rate at which carbon monoxide replaces oxygen from combination with human hemoglobin in solution and in the red cell. J. appl. Physiol. *11* (1957) 269–276.
28 Savoy, J., Michoud, M.-C., Robert, M., Geiser, J., Haab, P., and Piiper, J., Comparison of steady state pulmonary diffusing capacity estimates for O_2 and CO in dogs. Respir. Physiol. *42* (1980) 43–59.
29 Stainsby, W. N., and Otis, A. B., Blood flow, blood oxygen tension, oxygen uptake, and oxygen transport in skeletal muscle. Am. J. Physiol. *206* (1964) 858–866.
30 Wittenberg, B. A., and Wittenberg, J. B., Transport of oxygen in muscle. A. Rev. Physiol. *51* (1989) 857–878.
31 Wittenberg, B. A., and Wittenberg, J. B., Myoglobin-mediated oxygen delivery to mitochondria of isolated cardiac myocytes. Proc. natl Acad. Sci. USA *84* (1987) 7503–7507.
32 Zwart, A., Kwant, G., Oeseburg, B., and Zijlstra, W. G., Human whole-blood oxygen affinity: Effect of carbon monoxide. J. appl. Physiol. (Respir. envir. Exercise Physiol.) *57* (1984) 14.

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Research Articles

The structure of circinatin, a non-toxic metabolite from the plant pathogenic fungus *Periconia circinata*

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Summary. A new non-toxic metabolite, circinatin, has been isolated from culture filtrates of the fungus *Periconia circinata* grown under modified conditions which suppress the normal production of host-specific toxins. The structure of the new compound has been established as in **1** by combination of instrumental analysis and chemical degradation.
Key words. Milo disease of sorghum; *Periconia circinata*; circinatin; D-cyclolysine; D-aspartic acid; 3-(E-pent-1'-enyl)-glutaric acid.

The fungus *Periconia circinata* (Mangin) Sacc. produces host-specific toxins that are important pathogenicity factors causing disease symptoms on cultivars of sorghum susceptible to the fungus[3]. Abundant toxin production is observed when the pathogenic isolate is grown in 1-liter Roux bottles containing 200-ml or in 400-ml prescription bottles containing 100 ml of modified Fries' medium supplemented with 0.1% yeast extract[4,5]. From such cultures Wolpert and Dunkle[5] purified two PC-toxins which were characterized as peptides (MW < 2000) resistant to proteases and having aspartic acid as one of their constituents. We have now observed that when the fun-