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THE EFFECTS OF CATECHOLAMINES UPON THE METABOLISM OF FOREIGN COMPOUNDS AND UPON THE DISTRIBUTION OF PERFUSATE IN THE ISOLATED LIVER OF THE RAT

ALAN R. BOOBIS and GARTH POWIS*
Department of Pharmacology, Glasgow University, Glasgow, G12 8QQ, Scotland

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Abstract—Adrenaline and noradrenaline 6 μM, inhibited the rate of hexobarbitone metabolism by the isolated rat liver perfused at a constant flow rate, by a maximum of 62 and 54 per cent respectively. Neither catecholamine had any significant effect upon the metabolism of aniline. Hexobarbitone and aniline metabolism by rat liver slices was unaffected by either adrenaline or noradrenaline. It is suggested that the differential effect of adrenaline upon the metabolism of foreign compounds by the perfused liver is due to a partial hypoxia, which may be a result of the observed redistribution of perfusate away from some areas of the liver at the periphery of the lobes.

CATECHOLAMINES administered to rats have been reported to inhibit the metabolism of foreign compounds by the liver both *in vivo* and *in vitro*.[1,2]

We report the results of an investigation into the acute effects of catecholamines upon the metabolism of foreign compounds by the perfused rat liver, by liver slices and by the hepatic microsomal subcellular fraction. It is suggested that catecholamines lead to a partial hypoxia within the perfused liver, possibly due to the shunting of the perfusate away from the periphery of the lobes, resulting in a decrease in the metabolism of certain foreign compounds.

METHODS

Male albino rats of the Wistar strain, weighing between 200 and 250 g were anaesthetized with 1% halothane in O_2:N_2O, (1:3). The liver was isolated and perfused at a constant flow through the portal vein by a modification of the method of Powis.[3] The flow rate was set at 10 ml/min, usually giving a perfusion pressure of 10 cm H_2O, and changes in the perfusion pressure were measured on an Ether UP4 transducer. The perfusion medium consisted of 150 ml of washed aged human red cells, packed cell volume 25 per cent, in modified Krebs bicarbonate buffered saline, pH 7·4,[4] containing bovine serum albumin Cohn Fraction V powder 3 g/100 ml and glucose 0·15 g/100 ml. The perfusion medium was gassed with air containing 5% CO_2. The oxygen content of the medium was measured on a Lex–O_2–CON total oxygen analyser (Lexington Instruments). The liver was placed in a bath of liquid paraffin at 37° which supported the lobes and permitted an even perfusion. The viability of the preparation was assessed over 6 hr perfusion by measuring the rate of glucose liberation which

* Author to whome reprint requests should be addressed.

was 18 μmoles/g/hr, K^+ loss 10 μmoles/g/hr and bile flow 44 μl/g/hr. The pH of the perfusate remained constant at 7·4 and the ratio of lactate:pyruvate in the perfusate attained a steady value of 10:1. These values compare favourably with similar values reported for the rat liver both *in vivo* and *in vitro*.[5] The liver remained histologically normal after 6 hr perfusion when examined by both light and by electron microscopy.

After an equilibration period of 1 hr hexobarbitone sodium or aniline hydrochloride was added to the perfusate to give an initial concentration of 1 mM. Aliquots of perfusate were removed at various time intervals up to 4 hr. Hexobarbitone metabolism was determined according to the two pulse protocol described by Stitzel *et al.*,[6] and unmetabolized hexobarbitone assayed by the method of Remmer.[7] Aniline was extracted into 2 × 5 ml light petroleum (b.p. 60–80°) containing 1·5% isoamyl alcohol, re-extracted into 2 ml 2N HCl and assayed by the method of Bratton and Marshall.[8] Adrenaline or noradrenaline bitartrate was added to the perfusate to give and initial concentration of 6 μM, 85 min after the first hexobarbitone pulse and 5 min before the second pulse, or 1 hr after the addition of aniline.

The effects of adrenaline upon the distribution of perfusate within the liver were studied in two ways. First the liver was perfused with medium containing 10% (v/v) of the radio-opaque compound Conray 280 and X-radiographs of the liver were taken with a Newton Victor Model K, Type 5 X-ray Machine, at 30 keV, 3 sec exposure, on Type S X-ray film (3M Co). X-radiographs were taken before the addition of adrenaline and then at the peak of the pressure response to adrenaline. Secondly, the liver was perfused with medium containing 10% (v/v) Indian ink, particle size <3 μm (Gurr). Sections of liver were taken from different areas of the lobe after 2 min perfusion with the Indian ink, both in the absence of adrenaline and at the peak of the pressure response to adrenaline. The sections were fixed in Helly's fluid, blocked out and unstained 6 μm sections examined under the low power objective of a light microscope. The perfusate was not recycled in either of these studies.

Liver slices 0·4 mm thick were prepared as described by Umbreit *et al.*[9] and the metabolism of foreign compounds determined under the incubation conditions described by Cooper *et al.*[10] Substrates used were hexobarbitone 4 μmoles and aniline 2 μmoles. Aliquots of medium were taken at various time intervals up to 2 hr and unmetabolized hexobarbitone and aniline determined as described previously. The concentration of both substrates in the incubation medium followed an exponential decline. Metabolism has thus been expressed as the half life measured over 2 hr.

Hepatic microsomes were prepared by the method of Ernster *et al.*[11] and suspended in 0·25 M sucrose containing 0·05 M Tris pH 7·4, to give a protein concentration of 10 mg/ml. Protein was determined by the method of Lowry *et al.*[12] Drug metabolizing activity was determined over 30 min at 37° with the supporting system described by Schenkman *et al.*[13] Substrates used were hexobarbitone 0·6 μmole and aniline 0·2 μmole. Unmetabolized hexobarbitone and aniline were determined as described previously.

Drugs and chemicals used were adrenaline bitartrate, noradrenaline bitartrate (Sigma Chemical Co.), aniline hydrochloride (BDH), bovine serum albumin Fraction V Powder (Miles Laboratories), dibutyryl cyclic-AMP (Boehringer), halothane (I.C.I. Pharmaceuticals), hexobarbitone sodium, Conray 280 (May & Baker), papaverine hydrochloride (Merck) and Indian ink (Gurr).

RESULTS

Perfused liver: metabolic studies. Both adrenaline and noradrenaline added to the vascular perfusate at an initial concentration of 6 μM caused a rapid rise, within 2 min, in the perfusion pressure ($\pm$ S.E.M., n = 3), adrenaline by 13·7 $\pm$ 1·2 cm H_2O and noradrenaline by 11·4 $\pm$ 0·4 cm H_2O. The pressure then fell towards control levels, the half-life with adrenaline was 16·1 $\pm$ 1·2 min and with noradrenaline 23·5 $\pm$ 5·2 min. This fall in pressure is probably a reflection of the rapid metabolism of catecholamines by the liver.[14]

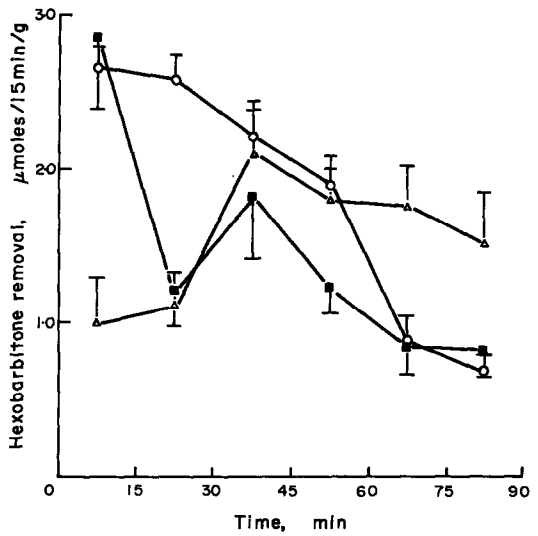

FIG. 1. The effect of catecholamines upon hexobarbitone metabolism by the isolated perfused rat liver. Hexobarbitone was present in 150 ml perfusion medium at an initial concentration of 1 mM. Control perfusions (○). Adrenaline (△) and noradrenaline (■) were added to the perfusion medium 5 min before the addition of hexobarbitone, at an initial concentration of 6 μM. Each point is the mean of four perfusions. Bars represent S.E.M. and for convenience are shown in one direction only.

The effects of adrenaline and noradrenaline upon the metabolism of hexobarbitone by the perfused liver are shown in Fig. 1. The two pulse protocol of Stitzel *et al.*[6] was employed but for convenience only the results of the second pulse are shown. Both catecholamines inhibited hexobarbitone metabolism within the first 30 min, by a maximum of 62 per cent ($P < 0.005$) for adrenaline and 54 per cent ($P < 0.005$) for noradrenaline. The half-life of hexobarbitone removal ($\pm$ S.E.M., n = 5) in control perfusions was 29·7 $\pm$ 2·0 min for the first pulse and 39·6 $\pm$ 4·3 min for the second pulse ($P < 0.05$). In the presence of adrenaline the half-life of the second pulse was increased to 87·1 $\pm$ 8·0 min ($P < 0.005$) and in the presence of noradrenaline to 72·4 $\pm$ 7·8 min ($P < 0.005$). The rate of metabolism was proportional to the concentration of hexobarbitone in the perfusate and fell throughout the duration of the pulse. The addition of adrenaline inhibited the rate of metabolism over the first 30 min and resulted in a comparatively high concentration of hexobarbitone remaining after 60 min. The rate of metabolism in these livers was thus higher after 60 min, when the effects of adrenaline had worn off, than in control livers.

Omitting Ca^{2+} from the perfusion medium reduced the vascular pressure response to adrenaline by 90 per cent and reduced the increased half life of hexobarbitone

removal caused by adrenaline to 55·2 ± 11·0 min (n = 3), a value not significantly different from the control value (P > 0·05). The omission of Ca^{2+} itself had no effect upon the half life of hexobarbitone removal during the first pulse being 34·0 ± 2·6 min (n = 3, P > 0·05).

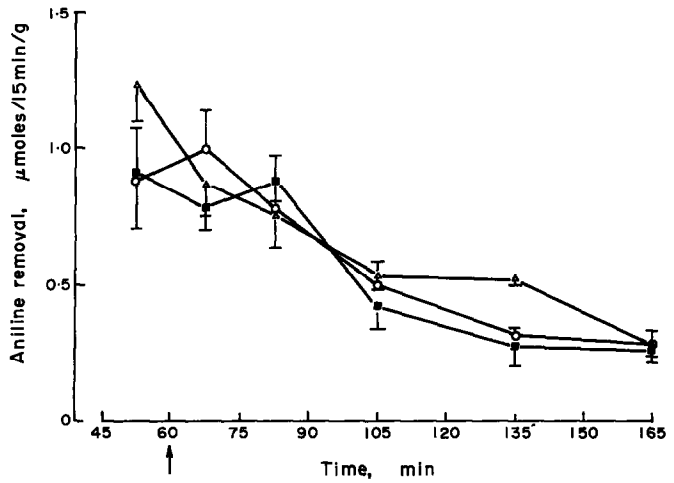

Fig. 2. The effect of catecholamines upon aniline metabolism by the isolated perfused rat liver. Aniline was present in 150 ml perfusion medium at an initial concentration of 1 mM. Adrenaline (△) and noradrenaline (■) were added to the perfusion medium after 60 min (↑) at an initial concentration of 6 μM. Control perfusions (○). Each point is the mean of four perfusions. Bars represent S.E.M. and for convenience are shown in one direction only.

The effects of catecholamines upon the metabolism of aniline are shown in Fig. 2. Adrenaline and noradrenaline were added to the perfusate after the initial rapid fall in the rate of aniline disappearance, on a more linear portion of the curve. It is probable that this initial rapid disappearance represents the removal of aniline by a non-metabolic process, possibly by tissue binding. Hydroxylated metabolites of aniline could not be detected in the perfusate until after 60 min. SKF-525A 2×10^{-4} M, an inhibitor of drug metabolism,[15] added to the perfusion medium had no effect upon the initial phase of aniline disappearance but inhibited the slower phase of disappearance by 54 per cent. The half-life of aniline removal (± S.E.M., n = 4) in control perfusions was 101·5 ± 6·8 min, in the presence of adrenaline 89·1 ± 3·9 min (P > 0·05) and in the presence of noradrenaline 100·0 ± 11·8 min (P > 0·05).

In the presence of papaverine 80 μM, an inhibitor of phosphodiesterase[16] the effects of adrenaline upon the removal of hexobarbitone by the perfused liver were not potentiated, as might have been expected if adrenaline was exerting its effects through cyclic-AMP. Adrenaline was still without effect upon the removal of aniline by the perfused liver. Although reported to inhibit the contraction of vascular smooth muscle[17] papaverine actually prolonged the pressure response to adrenaline by the perfused liver.

Perfused liver; blood distribution studies. X-radiographs of the liver perfused with medium containing Conray 280 revealed an even distribution of the contrast medium. Conray 280 itself caused an increase in the perfusion pressure of around 6 cm H_2O. The presence of adrenaline resulted in a marked alteration in the distribution of contrast medium with non-perfused areas at the periphery of the lobes.

TABLE 1. HEXOBARBITONE AND ANILINE METABOLISM BY RAT LIVER SLICES

	Half-life (min)*			
	n	Hexobarbitone	n	Aniline
Control	3	81·7 ± 5·6	6	84·4 ± 10·6
Adrenaline 10^{-3} M	4	91·3 ± 7·4	6	77·5 ± 7·0
Noradrenaline 10^{-3} M	3	99·6 ± 18·1	5	88·2 ± 5·6
Dibutyryl cyclic-AMP 10^{-3} M	3	131·7 ± 13·8†	—	—
SKF-525A 10^{-3} M	3	362·8 ± 7·5‡	—	—

* Values expressed are ± S.E.M.
† P = <0·05, ‡ P = <0·01 when compared to control value.

Changes in the distribution of perfusate produced by adrenaline were apparent to the naked eye, with small petechiae over the surface of the liver and local blanching. Perfusion with medium containing Indian ink had no effect upon the perfusion pressure and histological examination revealed the changes shown in Fig. 3. Sections of liver taken deep from the centre of the lobe showed no alteration in the distribution of Indian ink at the peak of the vascular pressure response to adrenaline. Many sections taken from the periphery of the lobe however showed a relatively poor perfusion in the presence of adrenaline. An examination of the sections under the high power objective revealed that the carbon grains were trapped within the sinusoids and had not been taken up by the cells of reticuloendothelial system. The distribution of carbon grains was thus related to the extent of perfusion of the area under view. Because the liver was being perfused at a constant flow rate and since adrenaline produced no marked alteration in the volume of the liver, the pattern of the distribution of the Indian ink represents a shunting of perfusate away from the periphery of the lobes.

Liver slices. To investigate further the possibility that catecholamines were exerting their effects in the perfused liver by altering the distribution of perfusate, the effect of catecholamines upon the metabolism of foreign compounds by liver slices was studied. Liver slices by their nature are independent of changes in the distribution of perfusate. The results are shown in Table 1. Neither adrenaline nor noradrenaline at concentrations up to 10^{-3} M had any significant effect upon the metabolism of hexobarbitone or aniline. Dibutyryl cyclic-AMP 10^{-3} M, increased the half life of hexobarbitone removal by 61 per cent. This is in agreement with the findings of Weiner *et al.*[18,19] SKF-525A an inhibitor of drug metabolism[15] was included to test the integrity of the system and produced a marked inhibition in hexobarbitone metabolism.

TABLE 2. HEXOBARBITONE AND ANILINE METABOLISM BY THE MICROSOMAL SUBCELLULAR FRACTION

	nmoles/30 min/mg protein			
	n	Hexobarbitone	n	Aniline
Control	4	49·6 ± 2·6	6	5·9 ± 0·5
Adrenaline 10^{-3} M	4	54·6 ± 3·7	6	7·1 ± 1·2
Noradrenaline 10^{-3} M	5	58·3 ± 6·2	4	6·9 ± 0·6

Microsomal subcellular fraction. Neither adrenaline nor noradrenaline at concentrations up to 10^{-3} M had any significant effect upon the metabolism of hexobarbitone or aniline by the hepatic microsomal subcellular fraction (Table 2).

DISCUSSION

The isolated perfused rat liver maintained its functional and histological integrity for up to 6 hr. The perfusion medium was gassed with air containing 5% CO_2, to avoid the degenerative changes reported by Abraham *et al.*,[20] when using oxygen containing 5% CO_2. The lower oxygen tension had no deleterious effects upon the liver as measured by the normal criteria of viability. There still existed the possibility that because of the low haematocrit of the perfusion medium the liver might be receiving less oxygen than *in vivo*. The oxygen content of the perfusion medium was 8·6 vol per cent. Although no data could be found for the rat, in unoperated dogs the oxygen content of arterial and mixed venous blood is 10·5 and 5·9 vol per cent respectively.[21] The portal vein supplies 80 per cent of the blood flow to the liver in the dog[22] which means that the liver receives blood with a mean oxygen content of 6·8 vol per cent. The perfused rat liver therefore appears to be receiving medium with a higher oxygen content than *in vivo* and cannot be considered to be hypoxic.

Perfusing the liver at a constant flow rate mimics the situation of the liver *in vivo*. Adrenaline administered directly into the circulation of an unanaesthetized rat produces a local vasoconstriction but no decrease in the blood flow to the liver. There may even be a reflex increase in hepatic blood flow initiated by a rise in blood pressure.[23]

Adrenaline and noradrenaline produced a marked inhibition in the metabolism of hexobarbitone by the perfused liver. One possible explanation is that catecholamines were exerting their effects upon the metabolism of foreign compounds through cyclic-AMP which has been reported to inhibit hexobarbitone metabolism in both the perfused rat liver and in liver slices.[18,19] Catecholamines however had no effect upon the metabolism of aniline by the perfused liver. The major route of aniline metabolism is by aromatic hydroxylation,[24] which is also inhibited by cyclic-AMP.[25] Papaverine, an inhibitor of phosphodiesterase[16] failed to potentiate the effects of adrenaline upon the metabolism of either aniline or hexobarbitone. Catecholamines also failed to inhibit hexobarbitone metabolism by liver slices. It is unlikely therefore that catecholamines were exerting their effects upon the metabolism of foreign compounds directly through cyclic-AMP. Whilst many of the effects of catecholamines are undoubtedly mediated through cyclic-AMP,[26] in comparison to other agents such as glucagon, catecholamines produce a relatively small increase in the levels of cyclic-AMP within the parenchymal cell,[27] which under physiological conditions are probably too low to exert a significant effect upon the metabolism of foreign compounds.

Adrenaline leads to a redistribution of perfusate away from the periphery of the lobes in the perfused liver, which might result from vasoconstriction at the level of the portal venous tree[28] or at the level of the sinusoids.[29,30] Daniel and Prichard[31] have reported that adrenaline administered to rats *in vivo* leads to a restricted intrahepatic circulation of the portal venous blood with an increased transit time across the liver. It is only possible however to show that there is a redistribution of blood

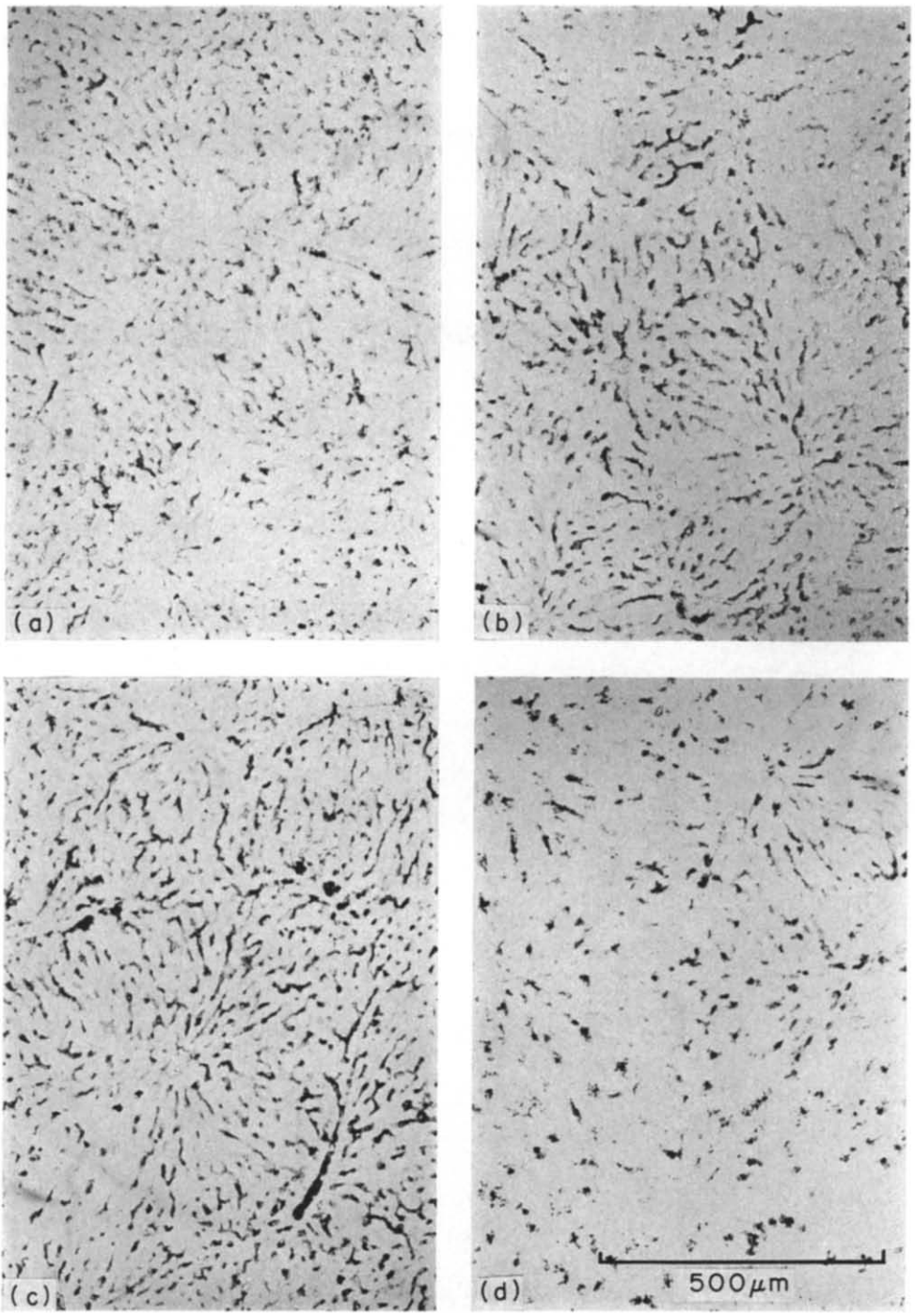

FIG. 3. Changes caused by adrenaline in the distribution of perfusate containing 10% (v/v) Indian ink, in the isolated rat liver. (a), unstained sections taken from the centre and (b), from the periphery of the lobes in control perfusions. (c) Unstained sections taken from the centre and (d), from the periphery of the lobes at the peak of the pressure response to adrenaline 6 μM.

flow rather than just a restricted intrahepatic circulation, when the liver is perfused at a constant flow rate as in the present study.

Hexobarbitone metabolism is inhibited by low oxygen tensions in both the perfused liver[32] and the microsomal subcellular fraction.[33] The metabolism of aniline by the microsomal subcellular fraction is unaffected by low oxygen tensions.[33] Adrenaline might lead to a partial hypoxia in the perfused liver by directing perfusate away from certain areas at the periphery of the lobes. This could then explain the effects of adrenaline upon the metabolism of hexobarbitone and the lack of effect upon aniline metabolism. Adrenaline also leads to an increased oxygen consumption by the liver[34] and might produce an overall relative hypoxia. This might explain why adrenaline produces a greater inhibition of hexobarbitone metabolism than noradrenaline. Adrenaline has no effect upon the metabolism of hexobarbitone by liver slices, which are independent of effects upon the distribution of perfusate and hypoxia. Omitting Ca^{2+} from the medium perfusing the liver abolishes the vascular pressure response to adrenaline and also partially prevents the inhibitory effects upon hexobarbitone metabolism. Ca^{2+} is however also necessary for the activation of adenyl cyclase by adrenaline[35] and omitting Ca^{2+} might prevent an increased metabolism leading to an elevated oxygen consumption.

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