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Intracapillary Oxyhemoglobin Saturation in Malignant Tumours with Central or Peripheral Blood Supply*

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Abstract—*Solid tumours of DS-Carcinosarcoma show two different types of vascular patterns. Central blood supply and drainage are evident in tissue-isolated tumours in the rat kidney, but peripheral vascularization is found within tumours growing subcutaneously (s.c.). The oxygenation of both tumour types has been assessed by measurement of the intracapillary oxyhemoglobin saturation using a cryophotometric micromethod. In common with other solid tumours, measurements in tumour capillaries with diameters between 3 and 8 μm reveal that very low oxyhemoglobin saturation values predominate during normal respiration. However, comparison of tumours at the same stage of growth indicates significantly higher saturation values in s.c. growing tumours than in tissue-isolated ones. It is concluded that the radiosensitivity must be higher in tumours with peripheral blood supply and drainage. In addition, respiration of pure oxygen should increase the radiosensitivity of tumours with peripheral vascular pattern more than that of tumours with central blood supply.*

INTRODUCTION

A LIMITED growth of new blood vessels and an inadequate terminal vascular bed are general features of solid tumour growth. The reduction of the vascular bed runs parallel with a widening of the vessel diameters, an increase in length and a broadening of the intercapillary distances. Thus, the nutritive vessels are stretched to supply a mass of tumour tissue many times larger than the original tissue. In addition, lacuna-like, sinusoidal and cystiform blood vessels which can no longer be drained lead to sluggish or static blood flow. Thrombosis follows and occludes the microvessels. Therefore, in some areas of malignant tumours blood does not circulate despite the presence of intact vessels. A considerable part of tumour blood passes through arteriovenous shunts instead of capillaries and, hence, is not involved in the nutrition of the tissue [1–5]. Thus great heterogeneity in regional blood flow exists in solid tumours.

Disturbances of microcirculation, limited convective and diffusive transport within malignant tumours during growth, and a diminished oxygen transport capacity of the blood as a result of progressive anemia cause a deterioration of oxygen supply to the cancer cells. This may be shown by polarographic measurements of O_2 partial pressures (pO_2) in tumour tissue using gold microelectrodes. The measurements reveal low mean tissue pO_2 values approaching zero levels as the tumours increase in size. The pO_2 histograms show frequency distributions which are shifted to very low pO_2 values if compared with pO_2 histograms of normal tissues [4–7]. These findings are substantiated by measurement of the intracapillary oxyhemoglobin saturation. It was shown that very low intracapillary oxyhemoglobin saturation values predominate in malignant tumours [8]. Marked regional differences can only be found in those areas where a sufficient vascularization still exists. Frequency distribution curves of oxyhemoglobin saturations are shifted to very low values in comparison with corresponding distribution curves of normal tissues [9]. These experiments provide evidence that tissue hypoxia and even anoxia occur as the tumour increases in size.

In spite of evident similarities between malignant tumours, many investigations have re-

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vealed heterogeneities in cell proliferation and in radiation response. Since the mean tissue pO_2 and the oxygen concentration, respectively [10, 11], are important determinants of tumour growth and of the resistance of tumours to radiotherapy the uneven distribution of radiosensitivity could be a result of differences in tumour oxygenation. Since the diffusion constants for oxygen in neoplastic tissue do not differ from those of normal tissues with similar water content [12, 13], and since respiration of malignant cells appears to be similar to normal cells [14], these differences must be attributed mainly to different arrangements of the nutritive vessels. Different arrangements of the nutritive vessels, however, depend on the implantation site of the tumours. Recently, Habighorst [15] has pointed out that in s.c. implanted tumours there is a concentric arrangement of the tumour vessels with a dense peripheral tumour vascularization around a more or less avascular tumour centre. In contrast, after intramuscular (i.m.) tumour implantation diffuse penetration of the tumour with vessels is observed. In order to explain differences in the radiation response of tumours to single large doses of radiation, Falk [16] has described two different types of vascular patterns, a peripheral one and a central one. However, only a part of the radiation experiments allows a firm conclusion. The aim of the present experiments is to study the oxygenation of tumours at different implantation sites by investigating the intracapillary oxyhemoglobin saturation. Therefore, a cryophotometric micromethod is applied to solid tumours of the same type but with different patterns of vascularization i.e., with a central or a peripheral blood supply and drainage.

MATERIALS AND METHODS

Experiments were performed on a total of 23 Sprague–Dawley rats of both sexes (strain F. W. 49 Biberach) with body weights ranging from 307 to 435 g. The animals were fasted overnight but allowed free access to water. They were anaesthetized by i.p. injection of sodium pentobarbital (25 mg/kg body weight) and placed on a heating table that maintained the body temperature at 37.5°C. Animals were allowed to breathe air spontaneously. Blood coagulation was prevented by injection of Heparin (350 USP-units/kg body weight). The left carotid artery was cannulated in order to monitor the mean arterial blood pressure (MABP) by means of a Statham pressure transducer, and for measuring the relevant respiratory gas parameters (blood gas analyzer type A3, Eschweiler, Kiel, W.-Germany) as well as the hemoglobin concentration (cyanhemoglobin method) and the hematocrit (micromethod, Hawksley). Oxygen saturation values in arterial blood (S_{O_2}) were obtained from the arterial pO_2 values by using the O_2 dissociation curve of Sprague–Dawley rats as measured by Bork *et al.* [19]. At the end of the experiment the tumour was removed from the host in order to determine its wet weight. Only tumours of approximately the same stage of growth were studied.

Tumours with central vascular patterns were obtained when ascites cells of the DS-carcinosarcoma were implanted into the kidney of rats where they remain tissue-isolated [6, 17, 18]. After an average of 10–13 days the tumour cells completely replace the kidney tissue, and the tumour mass is connected to the host by only a single afferent and efferent vessel, both penetrating the tumour at its base and branching arborescently. Vascularization from the surrounding tissue does not occur. Within young tumours only single, small islands of necrosis can be detected. They unite to form larger necrotic areas only in very late stages of growth.

In order to obtain tumours with a peripheral blood supply, ascites cells of the DS-carcinosarcoma were implanted s.c. into the thighs of rats. Several days after implantation tumours with larger central necrotic areas appear. Although in older tumours there are larger necrotic areas in the centre, peripheral tumour areas still remain well vascularized by the parasitic blood supply from the surrounding host tissue.

The cryophotometric micromethod of Grunewald and Lübbers [20, 21] was employed for the measurement of oxyhemoglobin saturation values (HbO_2) in tumour capillaries. Details concerning the validity of the method and the calibration are described elsewhere [21]. Therefore, only a concise description of the measuring procedure is given here. Cylindrical tissue samples (2 mm in diameter, 5 mm in length) were excised rapidly from the *in situ* tumour with special tongs precooled in liquid nitrogen. Thus, the oxyhemoglobin saturation is fixed by rapid freezing. All tissue specimens were taken from a depth of about 1–5 mm where the oxygenation of the red blood cells is not affected by the diffusion of atmospheric oxygen into the tissue. The removed tissue specimen was transferred into liquid nitrogen and mounted

on a special sample holder which fits into a cryomicrotome (Slee, Mainz, W.-Germany) where the tissue cylinder was sectioned into 15 μm thick slices at −60°C. Afterwards, each single frozen slice was put between two precooled coverglasses. A copper cap with a central hole for photometry is glued to one of the coverglasses. This prevents the tissue probes from warming when they are transported from the cryomicrotome to the microscope cryostat. In the microscope cryostat the frozen tissue slices were kept at a temperature of −100 ±0.25°C by cold nitrogen gas. The cryostat was evacuated; the vacuum during measurement was about 10^{-5} mmHg. For further details concerning the cooling and vacuum system see Vaupel *et al.* [9]. The cryostat was installed on the stage of a Zeiss-microphotometer (UMSP I), i.e., a double beam photometer comparing the absorbance of the sample with the absorbance of a grey standard. The spectra were recorded within the range of 520–620 nm. The recording time for one spectrum was 10 sec. A diaphragm of 2.5 μm in diameter was used throughout all experiments. Only erythrocytes in tumour capillaries between 3 and 8 μm are detected. The data were recorded and stored on a digital magnetic tape and graphically displayed on an *x-y*-recorder. The spectra were evaluated by means of the multicomponent analysis of Lübbers and Wodick [22] who analyzed the measured spectra in terms of a linear combination of basis spectra. The spectra of the oxygenated, of the deoxygenated and of the artificial, dehydrated deoxygenated hemoglobin have to be considered. Further details concerning the intracapillary cryophotometric micromethod have been described elsewhere [9, 20, 21].

For statistical evaluation, the measured saturation values from tissue-isolated and s.c. growing tumours were grouped into classes of 5% saturation intervals. The relative frequency of their occurrence was plotted as a function of the saturation values. In order to determine statistical significance, the data of the HbO_2 saturation measurements were compared by the Mann–Whitney U-test. In addition, the cumulative frequencies of the saturation values were plotted.

RESULTS

Investigations were carried out on 10 tissue-isolated tumours with a mean wet weight of 5.0±2.0 g and on 13 s.c. growing tumours with a mean wet weight of 5.1±2.5 g. The relevant parameters of respiratory gas exchange throughout the experiments are listed in Table 1. Comparison of the data for the different tumour types reveals comparable conditions during the experiments with the exception of the mean hemoglobin concentration (Hb), and consequently, the mean oxygen content [O_2] of the arterial blood.

The frequency distribution curves of the measured saturation values (left panel) and the corresponding cumulative frequency curves of these data (right panel) for tissue-isolated tumours (solid line) and for s.c. growing tumours (dashed line) are shown in Fig. 1. The statistical evaluation of 453 saturation values from tissue-isolated tumour shows that 40% of the values lie between 0 and 5% saturation. Twenty-four per cent of the measured values are on zero level. Only 8% of the data exceed 50% saturation. The mean saturation value is 17%. The median is 9% oxyhemoglobin saturation, the modal class being 0–5% (see Table 2). With s.c. growing tumours, only 16% of the measured saturation values ($n=700$) are between 0 and 5%. Ten per cent of the data show zero level. Twenty-seven per cent of the obtained values are greater than 50% saturation. The mean saturation value amounts to 33%. The median is 22% saturation, the modal class being 0–5% (see Table 2).

If the single values of both distributions are compared by means of the Mann–Whitney U-test it can be stated that the oxyhemoglobin saturation values of s.c. growing tumours are significantly higher than those of tissue-isolated tumours ($P<0.001$).

DISCUSSION

(*a*) *Methods*

Since the cryophotometric micromethod has been discussed in detail elsewhere [9, 20, 21] only some important aspects will be mentioned here. Some modifications to the cryostat have improved the spatial resolution of the microscope and have enabled the determination of the oxygen saturation of single erythrocytes with a mean accuracy of ±4% without detecting any surrounding tissue [9]. Only red blood cells in tumour capillaries with diameters between 3 and 8 μm are subjected to measurement. As has been pointed out before, in malignant tumours, lacuna-like, sinusoidal and cystiform vessels with diameters up to 20 μm exist where blood does

Table 1. Parameters of respiratory gas exchange within the arterial blood of tissue-isolated tumours and of subcutaneously growing tumours during normoxia

	Tissue-isolated tumours	Subcutaneous tumours
art. pO_2 (kPa)*	13.1 ± 3.9	11.5 ±1.6
art. pCO_2 (kPa)	4.0 ± 2.8%	5.8 ±1.3
art. pH	7.31 ± 0.22	7.30 ±0.07
art. Hb (g/dl)	8.1 ± 3.5	13.3 ±1.5
art. Hct.	0.26 ± 0.10	0.41 ±0.03
art. S_{O_2} (%)	97 ±17	95 ±7
art. $[O_2]$ (ml/dl)	11.0 ± 4.8	16.7 ±2.4

*1 mmHg = 0.133 kPa.
Values are means ± S.D.

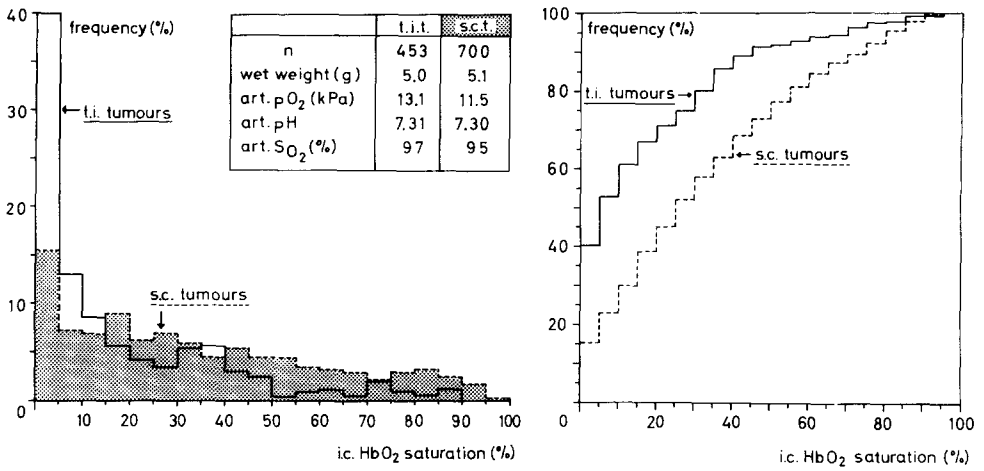

Fig. 1. Left panel: frequency distribution of measured oxyhemoglobin saturation values in tissue-isolated (solid line) and in subcutaneously growing tumours (dashed line). Right panel: cumulative frequency distribution of oxyhemoglobin saturation values in tissue-isolated (solid line) and in subcutaneously growing tumours (dashed line).

Table 2. Relevant parameters of the statistical evaluation of the measured intracapillary HbO_2 saturation values within tissue-isolated tumours and within subcutaneously growing tumours

	Tissue-isolated tumours	Subcutaneous tumours
Values in the range of 0–5% HbO_2 (%)	40	16
Values on zero level (%)	24	10
Values higher than 50% HbO_2 saturation (%)	8	27
Mean HbO_2 saturation (sat.%)	17	33
Median (sat.%)	9	22
Modal class (sat.%)	0–5	0–5

not circulate. The erythrocytes within these vessels do not take part in the respiratory gas exchange and have to be excluded from measurement. Were they to be taken into account, the distribution curves of the oxyhemoglobin saturation would be shifted to even lower values, since these blood cells have saturation values of zero. It is important to remove the tissue probes from comparable tumour sites. When sectioning the cylindrical probes, the orientation of the cylinders in relation to the tumour surface was arranged in a uniform manner. In this way, tissue slices of comparable locations within the tumours, were compared. Although tissue probes were taken only from a depth down to 5 mm from the surface the main tumour mass is subjected to measurement. If one assumes a 5 g tumour being a sphere with a diameter of 20 mm, the volume of a spherical shell with a thickness of 5 mm amounts to 88% of the volume of the whole sphere. Therefore, the interpretation of the experimental data is extended on the whole tumour mass.

(*b*) *Results*

It should be emphasized that only tumours of the same wet weight, i.e., at the same stage of growth, were compared. Figure 2 shows the frequency distribution of HbO_2 saturation values from s.c. growing tumours with different mean wet weights (Fig. 2, left panel). From the cumulative plot of these data it is obvious that saturation values in smaller tumours are higher than those obtained from larger tumours (Fig. 2, right panel; $P<0.05$). It is concluded that vascularization and, subsequently, the oxygenation of tumours deteriorate during growth, a result which supports findings of Jirtle *et al.* [23] and of Vaupel and Thews [7] who have investigated the tumour blood flow and the oxygen partial pressure in tissue, respectively, as a function of tumour growth.

Anemia is more pronounced in animals bearing tissue-isolated tumours than in those with s.c. growing tumours. Since anemia leads to a right shift of the O_2 dissociation curve, oxyhemoglobin saturation will decrease faster in the case of tissue-isolated tumours than with s.c. growing tumours when oxygenated blood enters the tumour capillaries. This effect, however, is not large enough to entirely explain the differences between the oxygenation states in both tumour types. If one assumes similar rates of oxygen consumption in both tumour types one would expect a lower oxygen saturation in tissue-isolated tumours where the oxygen transport capacity of the blood is smaller and exhausted faster than in s.c. growing tumours. However, measuring the oxygen consumption of DS-carcinosarcoma ascites cells, it could be unequivocally demonstrated that oxygen consumption is a function of oxygen supply (provided the supply conditions for other substrates, especially for glucose, remain constant, [24]). Hence, it must be assumed that oxygen consumption in s.c. growing tumour tissue is higher than in tissue-isolated tumours. This effect strongly counteracts the fact that the larger oxygen transport capacity of the blood in s.c. tumours is exhausted more slowly. Therefore, the differences in the hemoglobin content of tumour blood are not regarded as a main determinant of the differences in tumour oxygenation.

From the results presented here, it can be concluded that the pattern of vascularization plays a decisive role in oxygen supply to solid tumours. The vascular pattern of a tumour depends (i) on the implantation site and (ii) on the state of growth. Intracapillary oxyhemoglobin saturation values in s.c. growing malignant tumours with peripheral blood supply are higher than those in tissue-isolated tumours with central blood supply. In a previous investigation it could be shown that computed frequency distribution curves of tissue pO_2 values derived from intracapillary saturation measurements are in good agreement with distribution curves of tissue pO_2 values measured polarographically, i.e., intracapillary saturation values directly yield information about the oxygenation of the tissue [8]. As a consequence, radiosensitivity is expected to decrease with increasing tumour size and to be smaller for tumours with central blood supply than for tumours with peripheral vascularization. Since it was found previously that hyperoxia (i.e., respiration of pure oxygen) raises the tissue oxygen partial pressure only in areas where supply conditions are sufficient [4], one would expect that hyperoxia should improve radiosensitivity of tumours with a peripheral vascular pattern more than the radiosensitivity of tumours with a central blood supply.

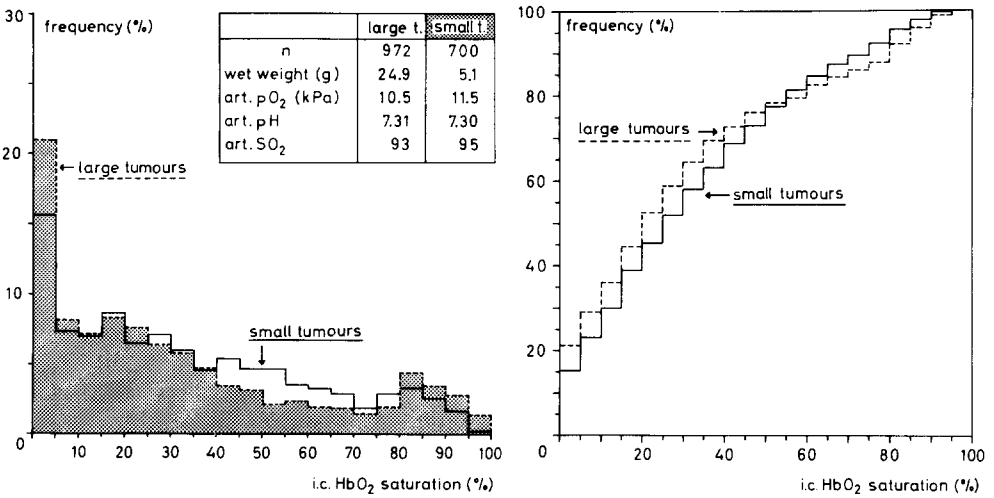

Fig. 2. Left panel: frequency distribution of measured oxyhemoglobin saturation values in subcutaneously growing tumours at different stages of growth. Right panel: cumulative frequency distribution of oxyhemoglobin saturation values in subcutaneously growing tumours at different stages of growth.

REFERENCES

1. I. F. Tannock, Population kinetics of carcinoma cells, capillary endothelial cells and fibroblasts in a transplanted mouse mammary tumour. *Cancer Res.* **30,** 2470 (1970).
2. P. M. Gullino, Extracellular compartments of solid tumours. In *Cancer.* (Edited by F. F. Becker) Vol. III, p. 327. Plenum Press, New York (1975).
3. P. Vaupel, P. Wendling and G. Thews, Kritische Sauerstoff- und Glucoseversorgung maligner Tumoren. *Dtsch. med. Wschr.* **101,** 1810 (1976).
4. P. Vaupel, Hypoxia in neoplastic tissue. *Microvasc. Res.* **13,** 399 (1977).
5. P. Vaupel, O_2 and glucose concentration and pH as limiting factors for cell proliferation in tumor tissue. International Symposium on Cell Proliferation and Differentiation in Normal and Neoplastic Systems. Essen (1977).
6. P. Vaupel, Atemgaswechsel und Glucosestoffwechsel von Implantationstumoren (DS-Carcinosarkom) *in vivo*. In *Funktionsanalyse biologischer Systeme.* (Edited by G. Thews) Vol. I. Steiner, Wiesbaden (1974).
7. P. Vaupel and G. Thews, pO_2 Distribution in tumor tissue of DS-carcinosarcoma. *Oncology* **30,** 475 (1974).
8. P. Vaupel, W. A. Grunewald, R. Manz and W. Sowa, Intracapillary HbO_2 saturation in tumor tissue of DS-carcinosarcoma during normoxia. *Adv. exp. Med. Biol.* **94,** 367 (1978).
9. P. Vaupel, R. Manz, W. Müller-Klieser and W. A. Grunewald, Intracapillary HbO_2 saturation in malignant tumors during normoxia and hyperoxia. *Microvasc. Res.* **17,** 181 (1979).
10. I. F. Tannock and G. G. Steel, Tumor growth and cell kinetics in chronically hypoxic animals. *J. nat. Cancer Inst.* **45,** 123 (1970).
11. I. F. Tannock, Oxygen diffusion and the distribution of cellular radiosensitivity in tumours. *Brit. J. Radiol.* **45,** 512 (1972).
12. J. Grote, R. Süsskind and P. Vaupel, Oxygen diffusion in tumor tissue (DS-carcinosarcoma) under temperature conditions within the range of 20–40°C. *Pflügers Arch. ges. Physiol.* **372,** 37 (1977).
13. P. Vaupel, Effect of percentual water content in tissues and liquids on the diffusion coefficients of O_2, CO_2, N_2 and H_2, *Pflügers Arch. ges. Physiol.* **361,** 201 (1976).
14. S. Weinhouse, Glycolysis, respiration and anomalous gene expression in experimental hepatomas: G. H. A. Clowes Memorial Lecture. *Cancer Res.* **32,** 2007 (1972).
15. L. V. Habighorst, Tierexperimentelle Untersuchungen zur Tumorvaskularisation. *Radiologe* **17,** 111 (1977).

16. P. FALK, Patterns of vasculature in two pairs of related fibrosarcomas in the rat and their relation to tumour responses to single large doses of radiation. *Europ. J. Cancer* **14,** 237 (1978).
17. P. M. GULLINO and F. H. GRANTHAM, Studies on the exchange of fluids between host and tumor. I. A method for growing 'tissue-isolated' tumors in laboratory animals. *J. nat. Cancer Inst.* **27,** 679 (1961).
18. P. VAUPEL, H. GÜNTHER, J. GROTE and G. AUMÜLLER, Atemgaswechsel und Glucosestoffwechsel von Tumoren (DS-Carcinosarkom) *in vivo*. I. Experimentelle Untersuchungen der versorgungsbestimmenden Parameter. *Z. ges. exp. Med.* **156,** 283 (1971).
19. R. BORK, P. VAUPEL and G. THEWS, Atemgas-pH-Nomogramme für das Rattenblut bei 37°C. *Anaesthesist* **24,** 84 (1975).
20. W. A. GRUNEWALD and D. W. LÜBBERS, Die Bestimmung der intracapillären HbO_2-Sättigung mit einer kryo-mikrofotometrischen Methode angewandt am Myocard des Kaninchens. *Pflügers Arch. ges. Physiol.* **353,** 255 (1975).
21. W. A. GRUNEWALD and D. W. LÜBBERS, Cryophotometry as a method for analyzing the intracapillary HbO_2 saturation of organs under different O_2 supply conditions. *Adv. exp. Med. Biol.* **75,** 55 (1976).
22. D. W. LÜBBERS and R. WODICK, The examination of multicomponent systems in biological materials by means of a rapid scanning photometer. *Appl. Optics* **8,** 1055 (1969).
23. R. JIRTLE, K. H. CLIFTON and J. H. G. RANKIN, Measurement of mammary tumor blood flow in unanesthetized rats. *J. nat. Cancer Inst.* **60,** 881 (1978).
24. W. MÜLLER-KLIESER, R. ZANDER and P. VAUPEL, Oxygen consumption of tumor cells suspended in native ascitic fluid at 1–42°C. *Pflügers Arch. ges. Physiol.* **377,** R 17 (1978).