CONTINUOUS EVALUATION OF P_{a,O_2} IN FISH: RECORDING AND DATA PROCESSING

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SUMMARY

1. In the eel (*Anguilla anguilla* L.), the dorsal and ventral aortas and the subhepatic vein were catheterized to provide an external circulatory shunt. P_{O_2} in the shunted blood was continuously recorded using a polarographic measuring device.

2. Spontaneous interruptions in the eel's ventilatory activity result in rapid changes in P_{a,O_2}. The period of these P_{a,O_2} oscillations is sometimes less than 1 min. However, the response time of the measuring system is so great under the conditions of these experiments that only slow variations of P_{a,O_2}, of a period greater than 5 min, are faithfully recorded.

3. In view of the impractibility of technical improvement in the recording system, a data processing method was developed to reconstitute the real physiological variations from the distorted experimental recordings.

4. Through this method of data correction, it is demonstrated that apnoeic or active ventilatory phases in the eel's breathing pattern are not triggered by constant low values of P_{a,O_2}. Other physiological applications are suggested.

INTRODUCTION

Several authors had observed that ventilation in the eel is spontaneously interrupted by periods of apnoea (Van Dam, 1938; Steen & Kruysse, 1964; Belaud, 1975). This ventilatory pattern results in rapid variations in blood pH, P_{CO_2} and P_{O_2}. Water breathing poikilotherms exposed to changes in enviromental factors (such as temperature, P_{w,O_2}, P_{w,CO_2}) may also exhibit large and sudden variations in blood characteristics (Dejours, 1972; Dejours, 1973; Dejours & Armand, 1973). Similar variations may be induced by changes in the internal milieu (e.g. injection of catecholamines (Peyraud-Waitzenegger, 1978)). These few examples demonstrate the need for continuous, accurate measurement of various blood characteristics.

Blood pH, P_{O_2} and P_{CO_2} have been continuously recorded in mammals, either by miniature catheterizable probes (Kreuzer, Harris & Nessler, 1960; Schuler, 1966), or by installing an external circulatory shunt permitting uninterrupted blood flow into a measuring chamber. In both cases the problem of response time of the probes must be taken into account; however, the latter technique has the advantage of allowing frequent calibration of the measuring system.

Implanting miniature electrodes in fish presents two difficulties: the size of the probes, which are of the same order as the blood vessels, and the necessity of re-calibrating the system, following temperature changes for example. At present, the chronic miniature electrode implantation method does not appear feasible, and only measurements made in an external circulatory shunt seem adequate for the continuous study of blood characteristics in fish.

The external circulatory shunt of the fish is characterized by a relatively small blood flow in comparison with that of large mammals, and by a much lower and more variable temperature (0–30 °C). These two factors result in a lengthening of the response time of the measuring electrode (Schuler, 1966), which would thus appear inadequate for following rapid variations of blood P_{O_2}.

The purpose of the present article is thus (1) to define the limits of validity of the recordings, continuously performed in an external circulatory shunt in the fish; and (2) in view of the impossibility of improving the technique itself, to provide a mathematical treatment of the recording, which should increase the validity and thus the range of application of this method. This technique will be illustrated by a study of the rapid variation in P_{O_2} in the arterial blood in a fish which exhibits intermittent ventilation. Adaptations of the techniques for other physiological applications are suggested.

MATERIALS AND METHODS

Ten eels (*Anguilla anguilla* L.) were used. Their weight ranged between 300 and 500 g. and the fish were maintained at all times at a temperature of 15 ± 1 °C.

The technique for chronic catheterization of the fish's ventral or dorsal aorta and the intestinal or subhepatic vein has been described previously (Smith & Bell, 1964; Randall & Stevens, 1967; Chan *et al.* 1967; Garey, 1969; Kirsch, 1971; Belaud, 1975; Payraud-Waitzenegger, 1978). Eels were anaesthetized with 3% ethyl carbamate in water, and, during surgery, the gills were irrigated with the air-equilibrated anaesthetic solution. The ventral and dorsal aortas and the subhepatic vein were fitted with catheters permitting blood withdrawal or re-injection. Electrodes recording ventilatory activity were attached to each gill cover (Belaud, Peyraud-Waitzenegger & Peyraud, 1971). In order to prevent blockage of the catheters, heparin (100 i.u. ml^{-1} in eel physiological solution) was administered through the implanted catheter; the total daily dose being 1000 i.u. kg^{-1}. The eels were allowed a recovery period of several days in an aquarium supplied with well-aerated water.

During the experiments, blood passed via the aortic catheter (ventral or dorsal) through the P_{O_2}-measuring cell and returned to the animal via the venous catheter. The thermostatted measuring cell (Radiometer D616) equipped with a polarographic electrode (Radiometer E5046) sensitive to oxygen partial pressure, was previously filled either with heparinized physiological saline (heparin concentration: 100 i.u. ml^{-1}) or with heparin-treated blood from other eels. No transfusion incompatibility was observed.

Blood flow was controlled by a peristaltic pump between the arterial catheter and the measuring cell. The constant blood flow of 0.47 ± 0.03 ml min^{-1} represents approximately 10% of the mean cardiac output for an eel of 300 g (Mott, 1957). The P_{O_2} measuring electrode was attached to a Radiometer PHM 71 analyser previously calibrated by means of blood samples from other eels equilibrated at the
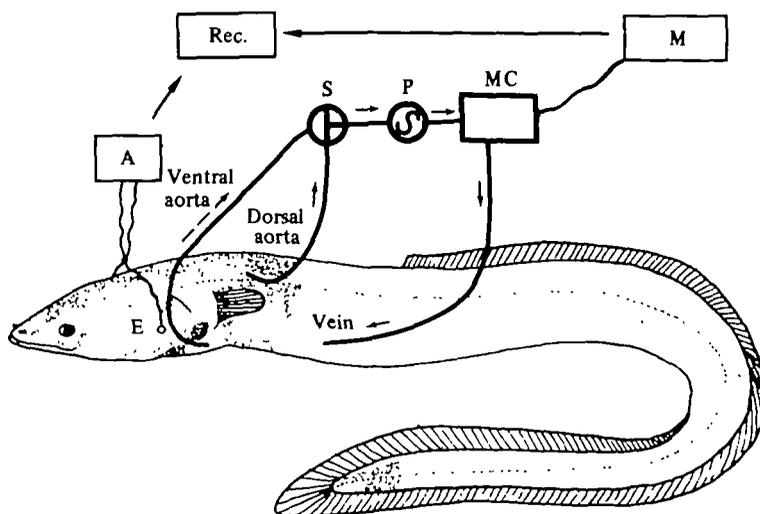
Fig. 1. Diagram of the measuring system. E, Electrodes for recording ventilation; A, amplifier; Rec., recorder; S, stopcock for selecting either dorsal or ventral aortic blood; P, peristaltic pump (0.47 ml min^{-1} constant flow rate); MC, measuring cell; M, P_{O_2}-meter.

same temperature using mixtures of known gaseous composition obtained by Wosthoff pumps.

Blood P_{O_2} and opercular movements were recorded simultaneously on a pen recorder and tape recorder. The data were later treated using a Hewlett-Packard 9825 A calculator.

RESULTS AND DISCUSSION

(1) *Characteristics of the blood P_{O_2} value measured via an extracorporeal arterio-venous shunt*

An example of the continuous recordings is shown in Fig. 2. The lower trace (curve 1) shows the apparent P_{a,O_2}-value in the dorsal aorta, and the opercular movements are shown in the upper trace. These recordings were obtained simultaneously from an eel weighing 420 g, maintained in air-equilibrated water at 15 °C.

In the course of this 8 min recording, the apparent P_{a,O_2} value ranged between 30 and 75 torr. The P_{a,O_2} trace shows more or less marked oscillations due to the intermittent ventilatory activity. Inspection of the ventilation and P_{a,O_2} traces reveals that a given P_{a,O_2} oscillation corresponds to a change in ventilatory activity which occurred approximately 65 s previously. This latency, a result of the transit time of the blood between the gills and the measuring chamber, depends on the geometry of the system, which thus must be determined for each experiment performed.

Fig. 3 shows what happens when, in an eel exhibiting a regular and continuous ventilatory pattern, the dorsal aortic blood is suddenly replaced by ventral aortic blood at the entry to the measuring system (three-way stopcock switch, Fig. 1). Prior to the change, the P_{a,O_2} value was stable at 88 torr; after about 3 min, the P_{a,O_2} value obtained was relatively constant at 3 torr. The latency (L) from the switch to
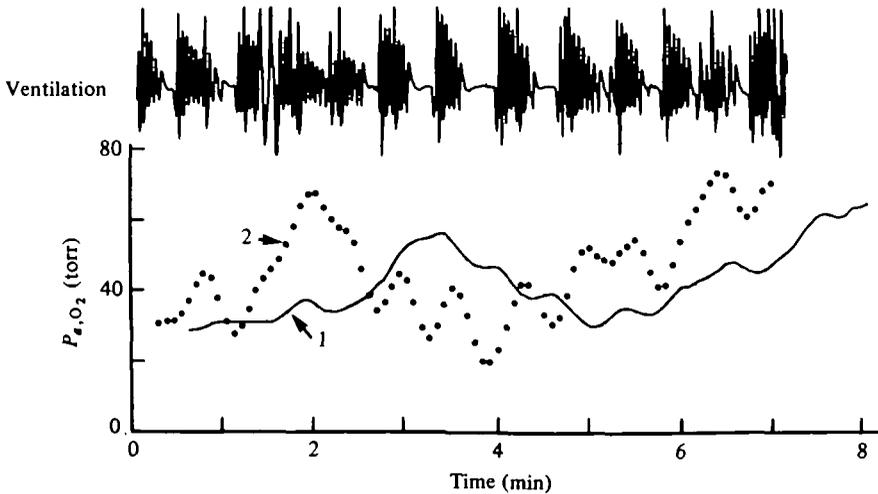
Fig. 2. Simultaneous records of the ventilatory movements (upper trace) and the apparent P_{a,O_2} (curve 1) from a 420 g eel breathing air-equilibrated water at 15 °C. In relation to the interrupted ventilatory pattern, curve 1 shows fluctuations occurring with a latency of about 65 s, corresponding to the delay in circulation along the catheters. Curve 2 is a plot of the calculated effective values for P_{a,O_2} at the implanted tip of the arterial catheter. Like the ventilatory activity, curve 2 shows marked oscillations, but it is clear that apnoeic or active ventilatory phases do not always coincide with a constant level of P_{a,O_2}.

the beginning of the response was 28 s in this experiment, less than that shown in Fig. 2, since the catheter was shorter. The solid line in Fig. 3, curve 1, represents the untreated recorded values which result from the quasi-instantaneous input change in the P_{O_2} value shown in the dashed curve 2. Comparison of curves 1 and 2 reveals that the time for a 95 % response was approximately 100 s which cannot be considered negligible in comparison with the period of the natural P_{a,O_2} oscillations shown in Fig. 2. Thus, Fig. 3 indicates the limits of validity of this measuring technique, which, in its present state, is only adequate for the study of very slow variations of P_{a,O_2} having a periodicity of approximately 5 min.

Data derived from experiments with ten different eels, using different catheters and electrodes equipped with propylene membranes of different ageing times show that the recorded P_{O_2} curve for a switch from dorsal to ventral aorta blood (Fig. 3) varied from one experiment to another. These curves, unique for each experiment, all show a characteristic exponential decline differing only in the time constant. The response time of the system, determined *in situ* by sudden substitution of venous for arterial blood entering the measuring cell, ranged between 90 and 130 s for 95 % response. This is due partly to the kinetics of the electrode, a function of temperature (Schuler, 1966), and partly to the kinetics of blood renewal in the measuring chamber, a function of the circulatory flow and the circuit volume. Here, the circulatory flow was held constant at 0·47 ml min^{-1} by the peristaltic pump and the temperature remained constant at 15 °C. Therefore the response time of the electrode depended primarily on the geometry and on the volume of the measuring chamber, length and width of catheters, etc., which were never identical from one experiment to another.

These experimental observations show that the kinetics of the response curve of
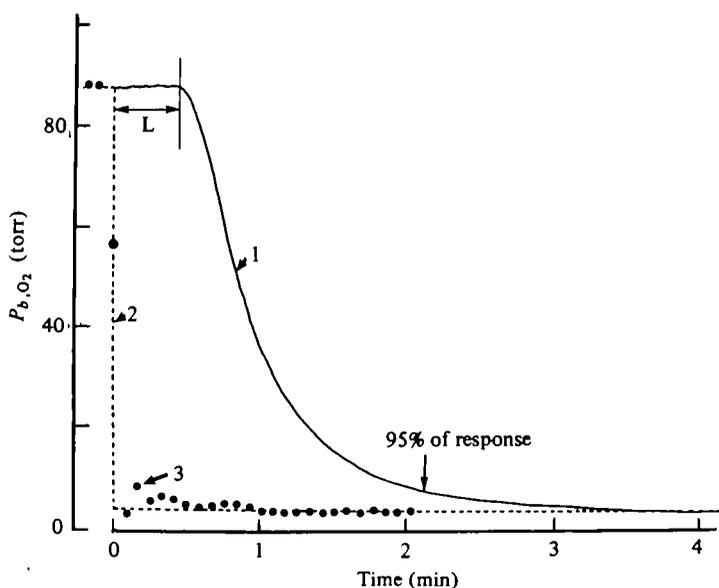
Fig. 3. Curve 1 is the record of apparent P_{O_2} obtained after an abrupt shift from arterial dorsal aortic blood to mixed venous ventral aortic blood entering the measuring system. After a latency period (L), the kinetics is exponential with a 95 % response time of about 100 s. The dashed curve (2) is the instantaneous change in P_{O_2} and the points (3) results from mathematical deconvolution method given in appendix. Differences between curves 2 and 3 are discussed in text.

system cannot be predicted in advance; rather, this curve must be established at the beginning of each experiment, checked periodically, and reestablished whenever elements of the circuit are modified: electrode, membrane, catheter, etc.

At first glance it appears possible to reduce the response time of the measuring electrode either by using thinner membranes or by constructing special rapid-response electrodes (Schuler, 1966). Several attempts have shown that, under such conditions, the response time may only be reduced by 30% at most, due to the existence of an unavoidable time lag: the flow rate of the external circulatory shunt. A supplementary experimental study of the dilution kinetics of a coloured fluid in the circuit was performed using dyes because a photosensitive cell has the advantage of responding without inertia. This study showed that, with a flow of 0·5 ml min^{-1} the irreducible time for dilution was approximately 85 s, an insufficient improvement. Classic-type electrodes were therefore used instead as they exhibit a greater stability with time.

In conclusion, the measuring system responds with a latency related to the dynamic characteristics of the external circulatory shunt. Continuous measures are unavoidably distorted to such an extent that rapid changes in the P_{a, O_2} value in fish cannot be studied. In the absence of any possibility of improving the technical aspect of the system, it was consequently necessary to develop a mathematical treatment of the recorded signal in order to reconstitute, from the experimental recordings, the real variations in P_{a, O_2} at the entry of the arterial catheter.

(2) *Data processing to obtain the actual values of P_{O_2} variations*
in the dorsal aorta blood

Two sets of data, the continuous recording of blood P_{O_2} from the experimental animal – the output of the measuring system – on the one hand, and the transient curve, which is the response of the system to a step-change in the P_{O_2} value of the blood entering the measuring cell, on the other hand, can be processed to yield the actual variations of P_{O_2} *in situ*. Details of the data-processing method are given in an appendix.

A transient curve, of the type illustrated in Fig. 3, is satisfactorily fitted by the form of the product of exponential terms (see appendix). The exponential terms can be interpreted as the quantitation of many physical phenomena occurring in the catheter (mixing of flowing blood layers), in the measuring cell (dilution of entering blood) or in the electrode (diffusion of oxygen through the membrane or through electrolyte). By analysis of the co-ordinates of points sampled on the transient curve, the exponent factor of each exponential term is calculated. The data treatment must start on a part of the P_{O_2} curves where the effective value of P_{O_2} is known and constant, i.e. at the end of a calibration operation. The calibration is carried out by flowing for more than 5 min a blood sample equilibrated at constant P_{O_2} (cf. methods). In this case, the final recorded P_{O_2} value can then be considered as the true value of P_{O_2} of the blood sample entering the electrode.

The suggested method for data processing was submitted to two successive control stages: (1) Fig. 3 shows an example of an experimental transient curve (curve 1), recorded after an abrupt switch from arterial dorsal aortic blood to venous ventral aortic blood entering the measuring cell. The real instantaneous change of P_{O_2} is represented by curve 2. The experimental curve 1 has been treated according to the method given in the appendix. The plotted points (curve 3), which are the calculated results, are closer to the theoretical curve 2. The residual errors which appear in Fig. 3 are interpreted as resulting from uncertainties in determining the exponent coefficients of the equation of the treated curve and in sampling the co-ordinates of points on the recorded curve. However, this test shows that a known step-change in P_{O_2}, modified by the measuring system, can be satisfactorily reconstituted by the data processing method suggested.

(2) Physical models were used to generate a P_{O_2} change of a known time course. In the experiment shown in Fig. 4, air-equilibrated water ($P_{w, O_2} = 156$ torr) was poured at a constant flow of $11 \cdot 5$ ml min^{-1} into a tank containing 2 l of water equilibrated at $P_{w, O_2} = 36$ torr. Curve 1 of Fig. 4 represents the calculated theoretical P_{O_2} change in the tank; and the experimental response curve is the solid line 2, which, after data processing, gave the dotted curve 3. The maximum differential between simultaneous points of curves 1 and 3 is 8% whereas the maximum differential between simultaneous points of curves 1 and 2 is 80%. Residual errors between curves 1 and 3 of Fig. 4 can be interpreted as resulting from uncertainties in sampling points on the recorded curves (see discussion above), as well as imperfections in performing the physical experiment. Sampling errors are minimized by using an automatic digital voltmeter.
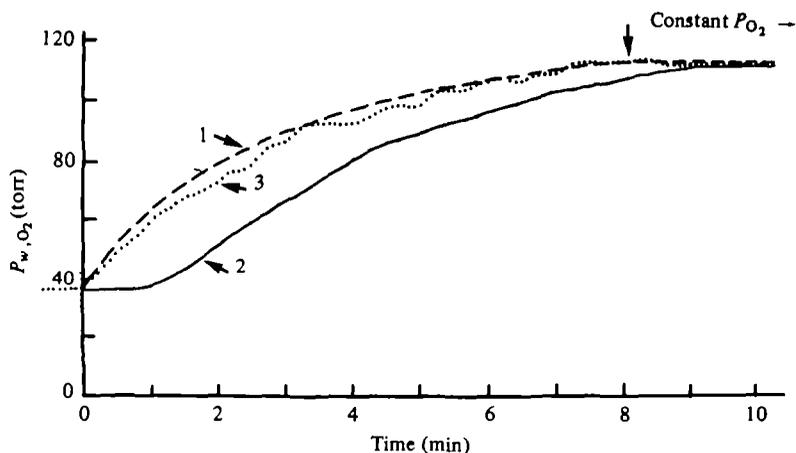
Fig. 4. Control of validity of the suggested deconvolution method with a physical model. The physical model generates in water a change in P_{O_2} represented by the dashed line 1; curve 2 is the experimental response of the measuring system and 3 are the points resulting from calculation.

(3) *Application of the data processing method*

The method for data processing described here has been used to investigate the relationship between fish's ventilation and arterial P_{O_2}. In Fig. 2, the continuously recorded experimental values for the eel's ventilatory activity and the apparent P_{a,O_2} (solid curve 1) are given together with the processed P_{a,O_2} values (curve 2). When the fish starts or stops ventilating, the effective P_{a,O_2} value is quite different from the value read on the meter. For the eel exposed to air-equilibrated water at 15 °C, the results do not support the view that active ventilation is always triggered at the same level of P_{a,O_2}.

The method can also be applied to investigations of rapid changes in blood pH or P_{CO_2} values. It will be useful in studies where the measuring system does not allow the instantaneous display of the true values of the physiological characteristics.

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APPENDIX

Data processing techniques

The unknown is the function $f(t)$ (in the present example, the changes in blood P_{O_2}, the input to the measuring system).

The data are: (1) the experimental curve $s(t)$, the output from the measuring system; (2) the previously determined transient response curve $r(t)$ (this is the output curve corresponding to an instantaneous step-change in the value of P_{O_2}); (3) the derivative $g(t) = [ds(t)/dt]$, the impulse response.

Using the impulse response, a relation may be established between the input function $f(t)$ and the output function $s(t)$.

Equation (1): $s(t) = g(t) * f(t),$

where the symbol * denotes convolution. The output function $s(t)$ results from the convolution of the input frunction $f(t)$ and the impulse response $g(t)$.

The following method of deconvolution permits the calculation of $f(t)$ from $s(t)$ and $g(t)$.

The operation of convolution, indicated symbolically in equation (1), is (Max, 1977):

Equation (2): $s(t) = \int_{u=0}^{u=a} g(u) f(t-u) \, du,$

where o and a are integration limits; u is an integration variable which covers the range (o, a).

In the particular case where the functions $s(t)$, $g(t)$ and $f(t)$ are known only at a finite number n of discrete points at constant spacing T in time, integration in equation (2) is effectively replaced by a sum. In this case, the value of $s(t)$ at the instant $t = nT$ is given by the equation (Brigham, 1974):

Equation (3): $$s(nT) = \sum_{j=0}^{j=n} Tg(jT)f([n-j]\,T).$$

This equation allows the calculations of $s(t)$ as the product of a convolution of $g(t)$ and $f(t)$. We are however intrested in the inverse process of deconvolution. Thus, we seek to transform equation (3) in such a way as to obtain a recurrence relation which allows us to obtain the value of $f(t)$ at each point in time from the values of $s(t)$ and $g(t)$. We proceed in two stages: (a) parametrization of $g(t)$; (b) deconvolution of $s(t)$ to obtain $f(t)$.

(a) Parametrization of g(t)

The experimental curves $r(t)$ (cf. Fig. 3) may be satisfactorily fitted by the analytical form:

Equation (4): $\quad r(t) = r_0 + [(r_\infty - r_0)\,(1 - e^{-(t/K_1)})\,(1 - e^{-(t/K_2)})\ldots(1 - e^{-(t/K_n)})],$

where $r = r(t)$ for $t = o$ is the initial value of P_{O_2}; r_∞ represents the value of $r(t)$ for $t \to \infty$; K_1, K_2, $K_3 \ldots K_n$ are the time constants for the respective exponential terms and are characteristic of the speed of response of the system. The physical significance of these exponential terms has been treated in the text. The values of K_1, K_2, $\ldots K_n$ can be obtained from calculations involving the coordinates of a few points on the experimental curve $r(t)$.

While the model described by equation (4) contains an arbitrary number of exponential terms, it is found in practice that the experimental curves can be fitted to within the experimental error by two or three exponents, additional terms being unreproducible and thus not significant. In order to simplify the following development, we shall retain only two such terms; the results for three terms will be stated without proof.

By differentiation of equation (4), and normalizing to $r_\infty - r_0 = 1$, we obtain the impulse response $g(t)$:

Equation (5): $\quad g(t) = \alpha_1 e^{-\alpha_1 t} + \alpha_2 e^{-\alpha_2 t} - (\alpha_1 + \alpha_2)e^{-(\alpha_1 + \alpha_2)t},$

where $\alpha_1 = 1/K_1$ and $\alpha_2 = 1/K_2$.

(b) Deconvolution of s(t)

Several different algorithms may be envisaged based on the use of various function transforms, The Fourier transform has been extensively used in this context, but we found that for our purpose, the Z-transform (Jury, 1964; Calvez, 1973) is particularly well adapted, because of its simplicity, and because of the discrete nature of our signal.

The Z-transform of function $f(n)$ is defined as:

Equation (6): $$F(z) = \sum_{n=0}^{n \to \infty} f(n)z^{-n}$$

and the Z-transformation of equation (3) can be written symbolically as:

Equation (7): $$S(z) = T\, G(z)\, F(z).$$

Use of the properties of Z-transformed functions allows us to calculate $G(z)$ from $g(t)$. Substitution of $G(Z)$ into equation (7) gives:

Equation (8): $$S(z) = T \cdot \frac{a_1 z^{-1} + a_2 z^{-2}}{1 + b_1 z^{-1} + b_2 z^{-2} + b_3 z^{-3}} \cdot F(z),$$

where, for clarity, we have introduced symbols a_1, a_2, b_1, b_3 and b_3 denoting the expressions:

$$a_1 = \alpha_1(e^{-\alpha_1 T} - e^{-(\alpha_1 + \alpha_2)T}) + \alpha_2 (e^{-\alpha_1 T} - e^{-(\alpha_1 + \alpha_2)T})$$

$$a_2 = \alpha_1 \cdot e^{-\alpha_2 T}(e^{-(\alpha_1 + \alpha_2)T} - e^{-\alpha_1 T}) + \alpha_2 e^{-\alpha_1 T}(e^{-(\alpha_1 + \alpha_2)T} - e^{-\alpha_2 T})$$

$$b_1 = (e^{-\alpha_1 T} + e^{-\alpha_2 T} + e^{-(\alpha_1 + \alpha_2)T})$$

$$b_2 = e^{-(\alpha_1 + \alpha_2)T}(1 + e^{-\alpha_1 T} + e^{-\alpha_2 T})$$

$$b_3 = -e^{-(2\alpha_1 + 2\alpha_2)T}.$$

Thus equation (8) may be rewritten as:

Equation (9): $$F(z) = \frac{S(z)}{T} \cdot \frac{1 + b_1 z^{-1} + b_2 z^{-2} + b_3 z^{-3}}{a_1 z^{-1} + a_2 z^{-2}}.$$

The inverse transform to equation (9), carried out according to the properties of Z-transform allows us to reconstitute the values of $f(t)$ at the instants T, $2T$, $3T$ $...nT$, from the equation.

Equation (10): $$f(n) = \frac{s(n+1) + b_1\, s(n) + b_2\, s(n-1) + b_3\, s(n-2) - T a_2\, f(n-1)}{a_1 T}.$$

This recurrence relation (which is mathematically rigorous) shows that once $g(t)$ is known, the value of $f(nT)$ at any instant nT may be calculated using the previous value $f([n-1]T)$ and four values from the curve $s(t)$.

In the case where the calculation is carried out using a model for $r(t)$ with three exponential terms (equation 4), we obtain the following recurrence relation:

(Equation 11): $$f(n) = \frac{s(n+1) + \sum\limits_{j=0}^{6} b_j s(n-j) - T \sum\limits_{j=1}^{5} a_j f(n-j)}{a_1 T}$$

where a_0 to a_5 and b_0 to b_6 are dependent on the time constants of exponential terms

This is apparently a problem in using the recurrence relations, in the case of equation (10) when $n < 2$, or in the case of equation (11) when $n < 6$, since then certain terms have negative indexes. In fact it is possible to show mathematically that all these terms may be simply omitted provided that we start the treatment on a part of the curve $s(t)$ where s is constant for a time T: $(s_1 = s_0)$. However, the very first point f_0 cannot be calculated and may only be obtained experimentally (see above).