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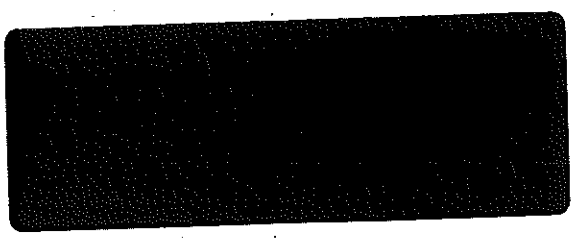

Mean transit time across the capillary membrane for different weights of FITC-dextran

G Rutili, P Hagander and K-E Arfors

ABSTRACT

Information on the mean transit time ($\bar{t}$) across the capillary membrane for different macromolecules may contribute to a better understanding of the transport mechanism involved. The plasma (C_p) and interstitial fluid (C_{if}) concentrations in the subcutaneous tissue of different molecular sizes of fluorescein labelled dextrans (FITC-dx) were determined as a function of time after a single intravenous injection. The experiments were performed in 35 rabbits. The plasma data were fitted using a two exponentials function. First order kinetics were then assumed for the transport across the capillary, $h(t) = A_3 \cdot e^{-k_3 t}$, and the interstitial fluid data was fitted by

$$C_{if}(t) = \int_0^t h(t-\tau) C_p(\tau) d\tau$$

This assumption gave good agreement with the experimental data. The $\bar{t}$ was given by the relation $\bar{t} = 1/k_3$. The data obtained indicates that two different mechanisms are involved in the transfer of macromolecules across the capillary, one related to molecular weights up to 40,000 and the other constant for all molecular weights. The calculated $\bar{t}$ is a weighted value of $\bar{t}$ for each of the two mechanisms.

Talk given at the 1st World Congress for Microcirculation, Toronto, June 15-20, 1975.

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Most of the present knowledge about the transfer of macromolecules across the capillary are mainly based on studies carried out on lymph. In these studies, usually, a lymph vessel has been cannulated and the concentration of different tracers is measured as a function of time, after intravenous injection of the tracer. These studies have primarily supplied information about steady state conditions. From the lymph to plasma concentration ratio at steady-state and from the lymph flow values information about the permeability properties of the capillary membrane have been evaluated.

This, however, gives little information about the mechanisms involved in the transport of these molecules. A better understanding of these processes could be achieved if additional information about transient events could be obtained. One of the parameters frequently used to describe these events is the mean transit time ($\bar{t}$). In the present studies, we have estimated the mean transit time from blood to the interstitium for different molecular weight dextrans.

The concentration of fluorescein labelled dextran (FITC-dx) in plasma and interstitial fluid was measured as a function of time after single intravenous injection of the tracer. Two different methods were used for the measurement of the concentration of dextran in the interstitium. With the first method, small interstitial fluid samples of 2 to 5 nanoliters volumes were obtained by micropuncture of the subcutaneous tissue of the hind-leg of rabbits. The concentration of FITC-dx in the interstitial fluid and in plasma was then measured in a microscope fitted with a photo-multiplier and dark-field condenser for fluorescence determination. The details of the measuring procedure and of the sampling procedure have previously been presented at the Aberdeen meeting of 1972.

The sensitivity of this method was, however, not sufficient to quantitate small changes in interstitial fluid concentration of FITC-dx for molecular weights higher than 20,000

because of the small absolute values of concentration, especially during the initial part of the experiment.

In order to increase the sensitivity of the method, direct in-vivo measurements of the amount of fluorescence in the tissue were performed. These studies required the use of a tissue which could be transilluminated. For this purpose, we have used the hamster cheek-pouch.

After nephrectomy, the pouch was exteriorized, fixed on a perspex table and prepared according to the method of Duling. In order to eliminate diffusion of the tracer from the tissue to the commonly used superfusing buffer solution, the pouch was superfused with nitrogen saturated paraffin oil at 37°. It was found that it was necessary to saturate the paraffin oil with nitrogen in order to lower the oxygen tension around the pouch, thus maintaining capillary blood flow in the cheek pouch.

The amount of fluorescence in a small area of tissue between capillaries was measured.

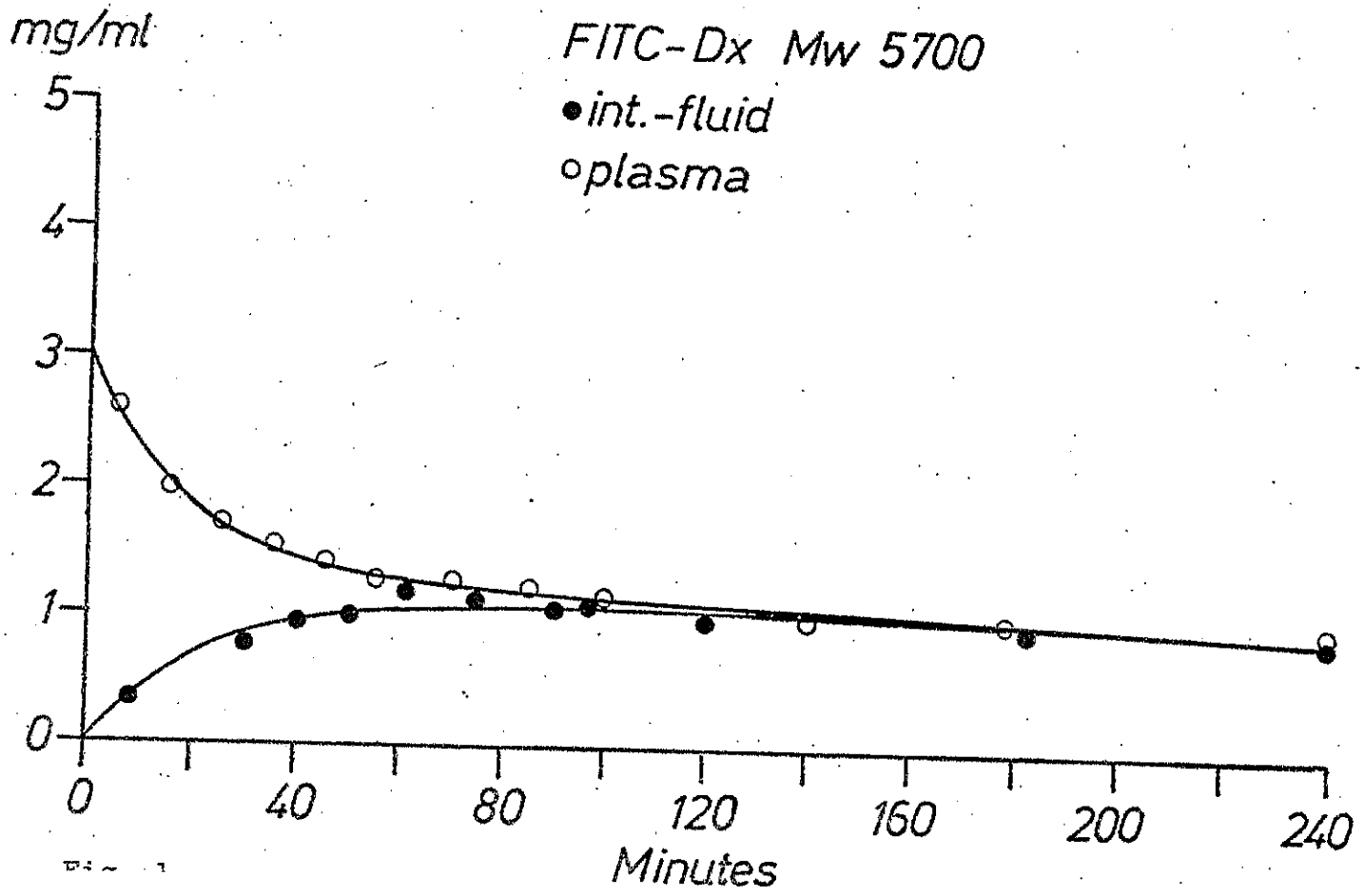
In order to calculate the mean transit time, certain assumptions were made:

1. The capillary flow is large as compared to the transcapillary flow and, thus, the plasma concentration constitutes the input to the system.
2. The interstitium is a well mixed compartment.
3. The transport of macromolecules across the capillary is described by first order kinetics.

The plasma data were fitted by a two-exponential function:

$$C_p(t) = A_1 e^{-k_1 t} + A_2 e^{-k_2 t}$$

First order kinetics were assumed for the transport across the capillary membrane, i.e.


mg/ml
FITC-Dx Mw 5700
•int.-fluid
°plasma
5
4
3
2
1
0
0
40
80
120
160
200
240
Minutes

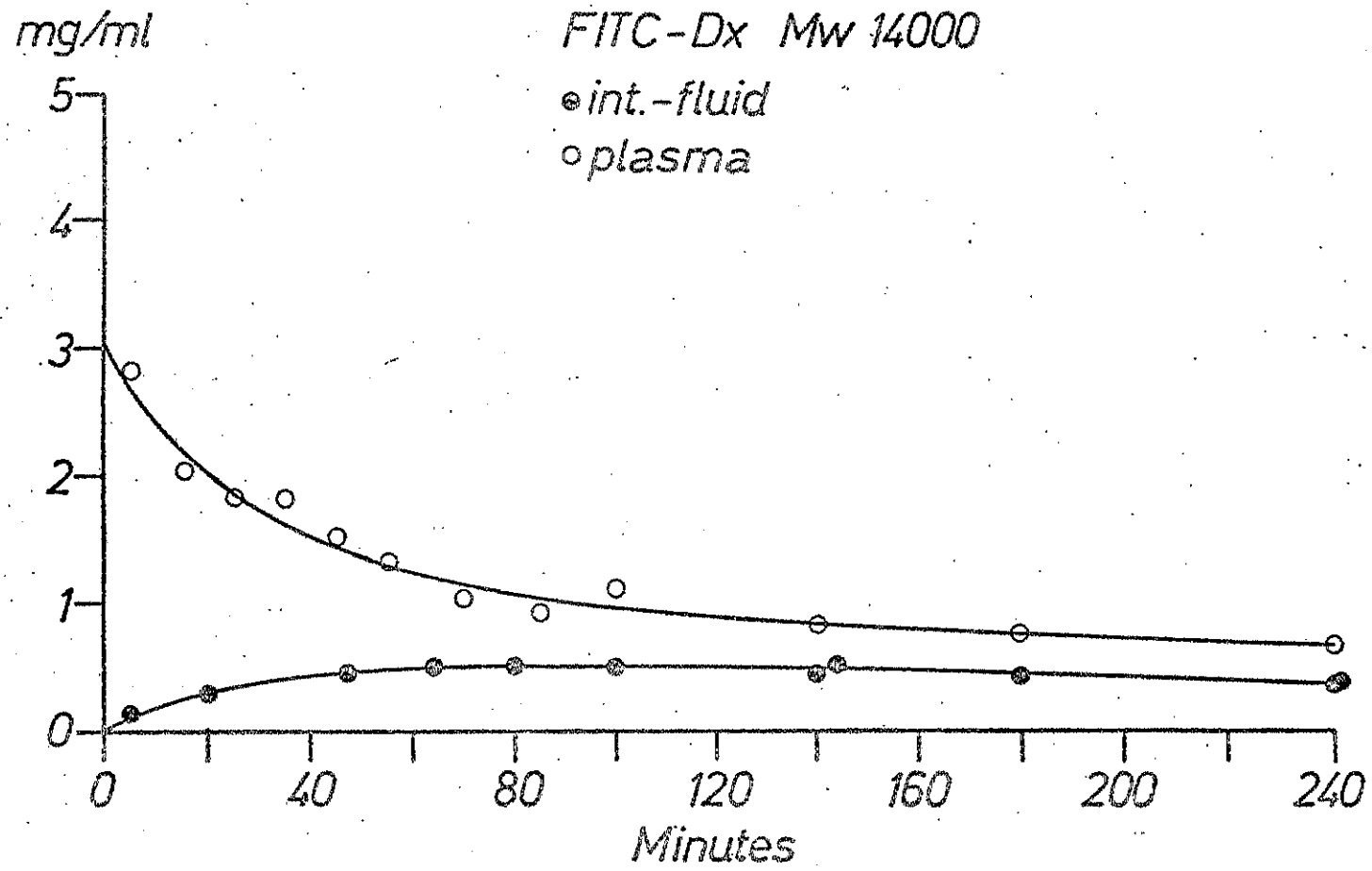

mg/ml
FITC-Dx Mw 14000
int.-fluid
plasma
5
4
3
2
1
0
0
40
80
120
160
200
240
Minutes

Fig. 2

$$h(t) = A_3 e^{-k_3 t}$$

and the interstitial fluid data fitted by

$$C_{if}(t) = \int_0^t h(t-\tau)\, C_p(\tau)\, d\tau$$

The $\bar{t}$ is given by the relation $\bar{t} = 1/k_3$.

A good fitting to the experimental data was obtained, as shown in fig. 1 and fig. 2, and it gave us no reason to assume a more complicated system than first order kinetics.

As a result of this calculation, the values for R and $\bar{t}$ in table I were obtained.

The two methods used for measurements of dextran in the tissue gave similar results for the same molecular weights (2300 and 19900). The same data has been plotted in fig. 3.

A similar behaviour for R and the inverse of $\bar{t}$ can be observed. Both decrease with increasing molecular size, reaching a constant value for molecular weights higher than 37,000.

The calculated values for $\bar{t}$ are somewhat different from those reported by Renkin in 1973, who found no consistent difference in $\bar{t}$ of 10,000 - 500,000 Mw of dextran. This difference is probably dependent on the fact that in Renkin's experiments, dextran fractions of relatively wide molecular weight distribution were used.

The Mw distribution of dextran in the lymph may, therefore, not be representative of the average Mw of the injected fraction. The results obtained indicate that these are two different mechanisms involved in the transport of macromolecules from blood to the interstitium. One, related to molecules up to Mw 20 - 40,000, and the other common for all molecular weights.

Mw	2 300	5 700	8 000	14 000	19 900	37 000	65 000	145 000
C_{if}/C_p (R)	1.0±0.04	0.98±0.15	0.78±0.09	0.59±0.06	0.35±0.05	0.10±0.06	0.10±0.04	0.98±0.08
Rabbit $\bar{t}$ (min)	33±17	51±13	71±18	105±37	186±24			
Hamster	25± 7				166±46	254±69	180±27	235±46

Table I

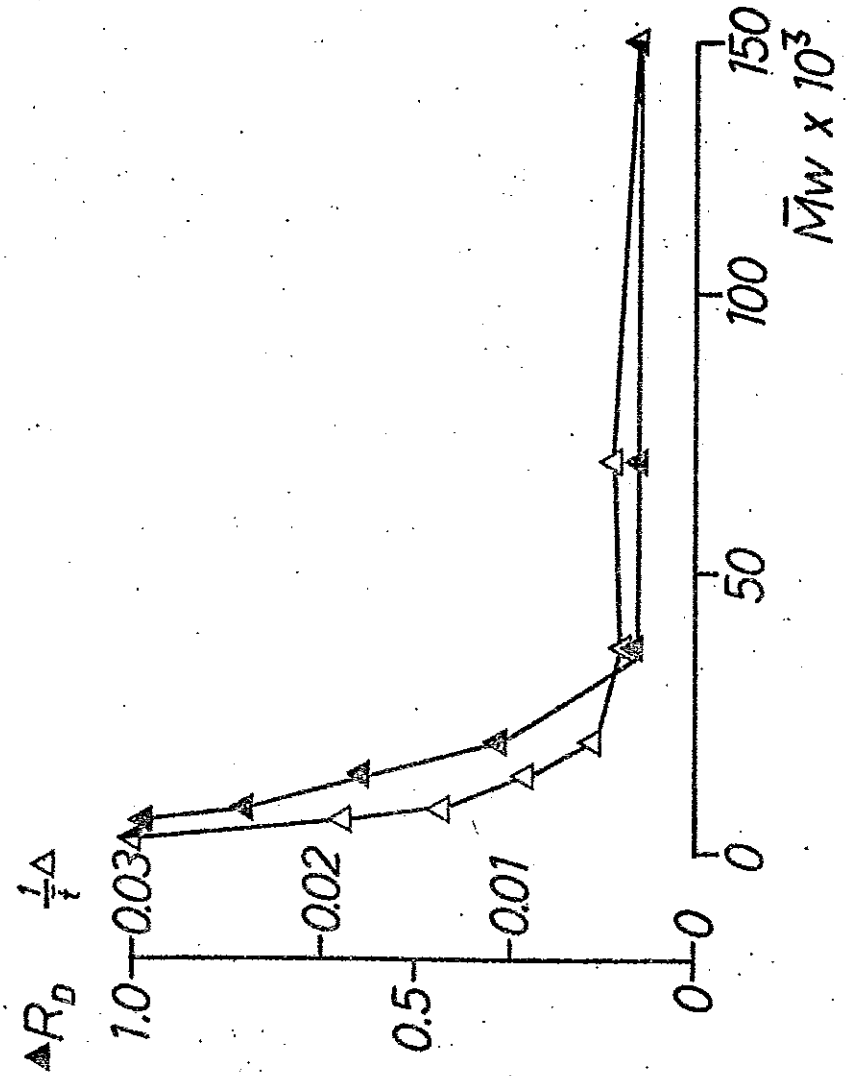


Fig. 3

The Mw dependent transport process has been described in the literature as a combination of filtration and diffusion through small pores of 40-50 A of radius. The Mw independent process has been explained in terms of pinocytotic transport or in terms of convection through large pores.

The ratio between R and $\bar{t}$ expresses the initial transcapillary plasma clearance per unit of interstitial distribution volume. Assuming identical Vd for the different Mw, the initial plasma clearance per 100 g of subcutaneous tissue was calculated (Table II and fig. 4).

It can be seen that it decreases with increasing Mw reaching a constant value of 0.03 ml/min for Mw higher than 37,000. This constant value should, therefore, represent the initial clearance through pinocytosis or convection via large pores.

By subtracting this value from the total clearance value, the transport through the small pores can be evaluated. A representation of the initial plasma clearance per 100 g tissue through the small pore system is shown in figure 5, where the broken line represent the slope for an unrestricted diffusive transport.

INITIAL PLASMA CLEARENCE

(ml/min/100 g)

2 300	5 700	8 000	14 000	19 900	37 000	65 000	145 000
1.81	1.20	0.66	0.33	0.11	0.024	0.030	0.025

Table II

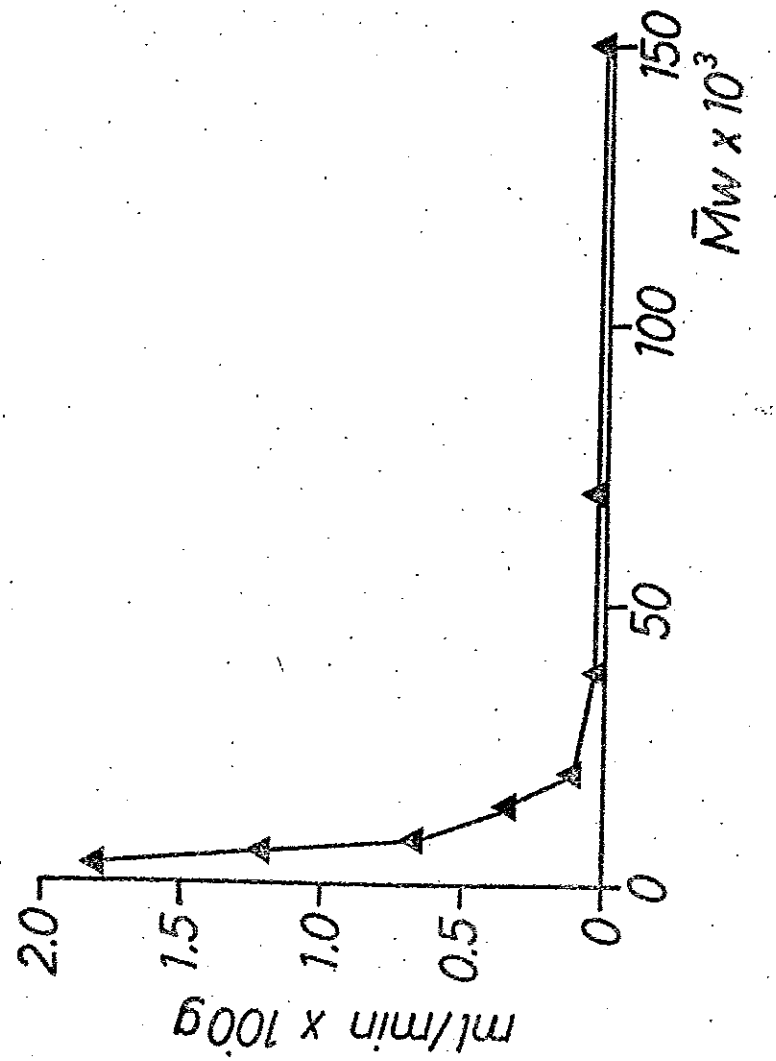


Fig. 4

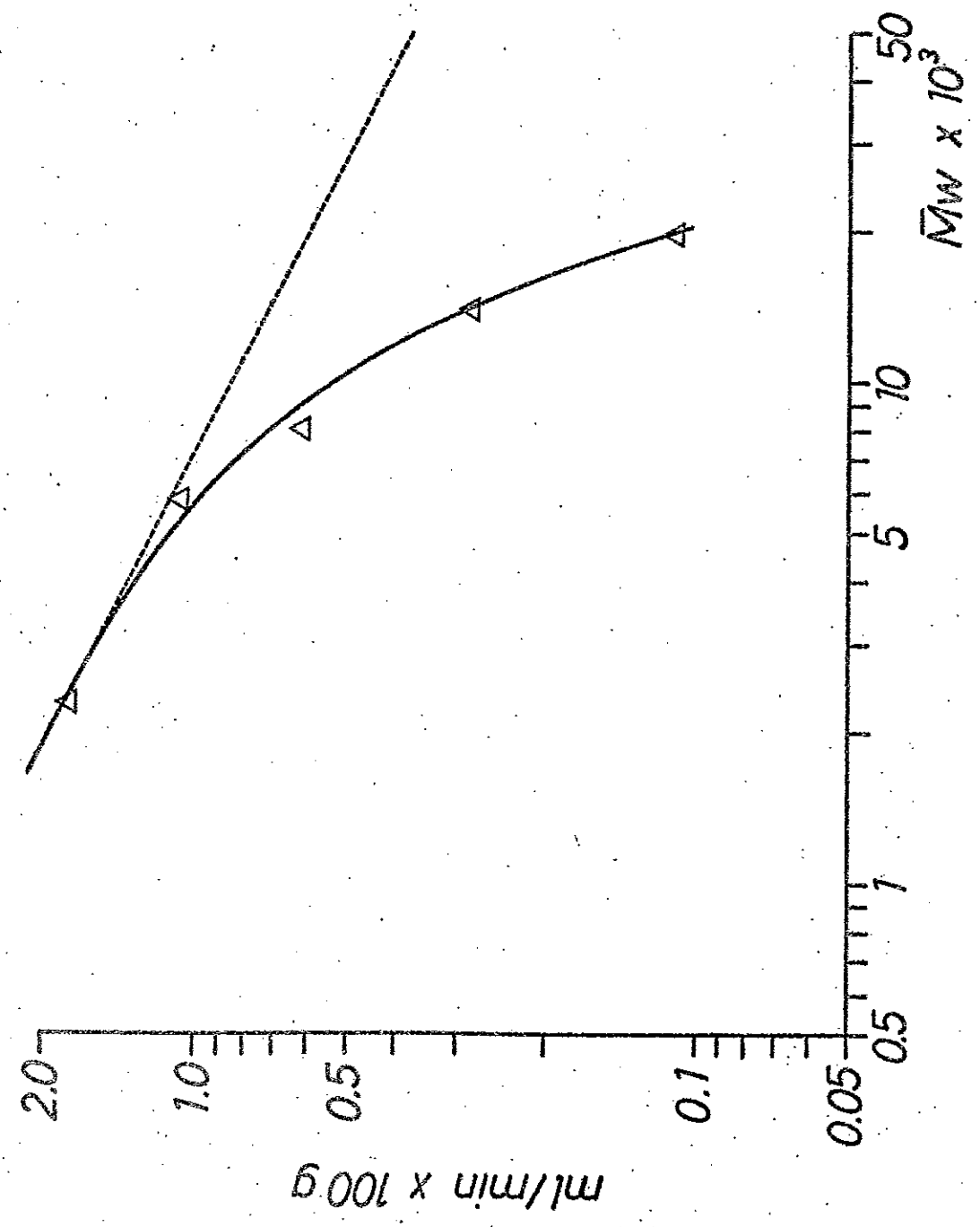


Fig. 5