

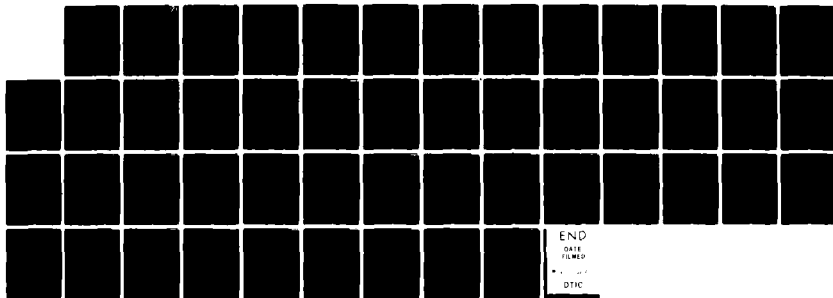
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AND WHOLE BLOOD MIXTURES(U) MASONIC MEDICAL RESEARCH
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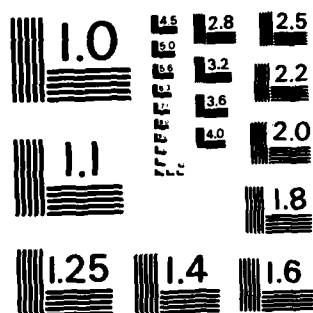
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The Biophysical Properties of Stroma Free
Hemoglobin and Whole Blood Mixtures

Final Report

L. C. Cerny

May 1981

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Utica, New York 13503

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) This is a preliminary investigation to establish and to develop the biophysical methodology for the evaluation of the use of stroma-free solutions as blood substitutes.		

Stroma-free hemoglobin solutions are promising plasma substitutes. They have satisfactory oxygen-carrying capacity, do not have any adverse effects on the renal function and offer some rheological advantages. An ideal plasma substitute should exert a sufficient colloidal osmotic pressure to maintain the circulating volume, should not increase the blood viscosity, especially at near stagnant conditions, should aid in the improvement of tissue perfusion and should not interact with the erythrocyte membrane or plasma proteins to cause aggregation. Stroma-free hemoglobin (SFH) meets these criteria. However, improvements are always taking place such as the amidination and pyridoxilation of the hemoglobin to increase the oxygen binding capacity, lipid encapsulation of the hemoglobin to prolong its in vivo retention, and the addition of suitable polymers to promote consistent rheological behavior. Because of the vast amount of work and interest in SFH and its modifications, it is extremely important to establish a data base through satisfactory model experiments as a means of recognizing and evaluating quantitatively the effects of any changes that may be made.

Rheological Properties:

The viscosity of the stroma-free hemoglobin (SFH) was determined in a Cannon-Ubbelohde Semi Micro Dilution Type

viscometer at 37°C. In all cases the kinetic energy correction factor was small enough to be neglected. The diluent was physiological saline at pH 7.4. The concentrations of these solutions were in the range from 2.5 to 30 gm/100 ml of Hgb. These data are shown graphically in (Fig.1) along with a Fig.1 similar set of measurements on albumin solutions. It should be noted that below the 10 gm/100 ml level, there is very little difference between the relative viscosities (η_{rel}) of the two solutions. The viscosity of the SFH solutions could be quantitatively represented by the generalized Mooney¹ equation,

$$\ln \eta_{rel} = \frac{[\eta]C}{1 - (k/v) [\eta]C} \quad (1)$$

In equation 1, $[\eta]$ is the intrinsic viscosity in dl/g, c, the concentration (g/dl) and k/v is a parameter which takes into account the molecular asymmetry and "crowding". From our data, $[\eta]$ was found to be 0.045 dl/g and k/v was 0.38. Using these values, it was possible to represent the experimental data to better than 5% deviation.

Simha² showed that the intrinsic viscosity of disc-like particle could be represented by

$$[\eta] = \frac{16f}{15 \tan^{-1} f} \quad (2)$$

3

where f was the axial ratio. If one uses the values given by Tanford³, f can be taken as 2.1 to 3.4, yielding an intrinsic viscosity between 0.0347 and 0.0493 dl/g which agrees well with our experimentally determined value.

To examine the effects of mixtures of SFH and plasma protein, viscosity measurements were made using two different SFH starting concentrations; one of 14.5 gm/100 ml and the other 7.25 gm/100 ml. These data are shown in (Fig.2) where the specific viscosity, η_{sp} , divided by the concentration is graphed versus the weight fraction of plasma protein. The specific viscosity is used because it represents the contribution to the viscosity due to the amount of polymer (SFH and/or protein) present above that of the solvent. The figure indicates a linear relationship. For comparison, mixtures of SFH (7.25 gm/100 ml) and albumin (6.5 gm/100 ml) are also graphed in (Fig.2).

Fig.2

Fig.2

The effect of shear rate on viscosity of SFH has not been definitely resolved. At high concentrations (i.e. above 15%), shear thinning may be a controlling factor in the micro-circulation. Some of these measurements are shown in (Fig.3A) and (Fig.3B) where the viscosity (centipoise) is graphed versus rate of shear (sec^{-1}). These measurements were made in a multi-bulb Ubbelohde viscometer and in a cone plate viscometer.

Fig.3A

Fig.3B

The non-Newtonian flow behavior of several mixtures of whole blood and SFH was examined. In (Fig.4), the viscosity at four different hematocrits is graphed versus the shear rate with a 6.1% SFH solution as the diluent. Below 30% Hct, the flow is Newtonian and independent of the shear. The effect of hemodilution is quite apparent and has a marked effect on the viscosity reduction. A similar experiment is illustrated in (Fig.5). In this investigation, the diluent is 14.5% SFH solution. The non-Newtonian flow region even extends to the 17.7% Hct mixture. This is in part due to the non-Newtonian behavior of this concentration of SFH which is indicated in (Fig.3). The hemodilution effect is not as pronounced as with the 6.1% SFH solution as illustrated with the samples of 53 and 42.4% Hct.

In addition to the flow properties, the rate of oxygen transport is of equal importance for cardiovascular and respiratory activity. As an indication of this property, it is normal to express the ratio of the hematocrit to the viscosity (Hct/η) as a function of the hematocrit (Hct) at a fixed shear rate⁴. This is shown in (Fig.6) for whole blood diluted with 6.1% SFH solution at the shear rates of 46 sec^{-1} and 23 sec^{-1} . These shear rates were chosen because they illustrated the greatest effect of this activity. The graph indicates that

a maximum does exist at a hematocrit of about 25%. Similar data are shown in (Fig.7). However in this graph, the diluent was 14.5% SFH solution. It is important to observe that the overall effect of this activity is reduced, there is no maximum in the curve and the effect is almost constant and independent of hematocrit. This indicates that the oxygen transport is apparently not directly increased with increasing SFH concentration. This is a complex relationship and intimately involved with the flow patterns in (Fig.4 and 5).

Fig.4
Fig.5

Osmotic Pressure:

The measurement of the colloidal osmotic pressure was determined with a modified Zweifach micro-osmometer. A constant temperature bath was used to control the temperature to better than $\pm 0.5^{\circ}\text{C}$. The concentration range extended to about 14 gm/100 ml. It was shown that the data could be represented by

$$\left(\frac{\pi}{c}\right)^{1/2} = \left(\frac{\pi}{c}\right)_0^{1/2} \left[1 + \frac{\Gamma_2 c}{2} \right] \quad (3)$$

In this equation, π represents the osmotic pressure at concentration C , and Γ_2 is the second virial coefficient which is a measure of the polymer-solvent interactions. A graph of $\left(\frac{\pi}{c}\right)^{1/2}$ vs. C results in a straight line. From the intercept, $\left(\frac{\pi}{c}\right)_0^{1/2}$ one obtains the number average molecular weight, M_n .

$$\left(\frac{\pi}{c}\right)_0 = \frac{RT}{Mn} \quad (4)$$

From the slope, one evaluates Γ_2 ,

$$\Gamma_2 = \frac{2 (\text{slope})}{\left(\frac{\pi}{c}\right)_0^{1/2}} \quad (5)$$

Equation (3) is used for the osmotic pressure rather than a plot of $\frac{\pi}{c}$ vs. C , because it eliminates the curvature which may lead to erroneous extrapolation procedures.

The osmotic pressure data are summarized in (Table 1) Table for three different temperature. Also included are the osmotic pressure data for other commonly used plasma expanders, hydroethyl starch (HES), dextran, polyvinylpyrrolidone (PVP) and albumin. The values of $\Gamma_2/[\eta]$ are presented because they should remain constant for a particular polymer series.

It is interesting to note the magnitude of the Γ_2 values for SFH in comparison to the series of other plasma expanders. The relative size indicates that the SFH solutions are approaching the ideal polymer solution, $\Gamma_2=0$.

Some data of mixtures with SFH and plasma proteins are shown in (Fig.8). In this graph the osmotic press/concentration (π/c) is graphed versus the weight fraction of plasma. Fig. Two SFH concentrations are presented, 7.25 gm/100 ml and 14.5 gm/100 ml. For comparison, some data with 7.0 gm/100 ml SFH and 6.5 gm/100 ml albumin mixtures are also graphed.

The osmotic pressure of mixtures of whole blood at different concentrations of SFH also present an interesting situation. These data are shown in (Fig.9). In this figure, the osmotic pressure in cm. of H₂O is graphed against the volume percent of SFH in the mixture. These determinations were made at four SFH concentrations, 3.5%, 7.0%, 9.5% and 15.2%. The values of the blood hematocrits are given in parenthesis on the graph. It is interesting to observe the maxima that occurs at the concentrations of 7.0% and greater at about 75 volume % of SFH. This would suggest that during infusion with SFH solutions the vessels in the circulatory system are in a hyper osmotic state relative to whole blood. This may have certain advantages in the treatment of shock.

Fig.

Erythrocyte Sedimentation Rate:

The erythrocyte sedimentation rate (ESR) studies were made in the automatic sedimentimeter⁵. This unit not only follows the settling process but it also electronically generates the first derivative of the curve, that is, the true rate of settling as a function of time. This is a new concept in ESR and has the potential of becoming a diagnostic indicator. A typical set of data are shown in (Fig.10) where both the settling curves and the first derivatives are taken directly from the sedimentimeter. A summary of data is presented in

Fi

(Table 2). It is our contention that the maximum velocity, $V_{\max}$ (cm/hr), is the most meaningful single parameter to document the ESR.

Table 2

A careful examination of (Table 2) indicates some interesting results. First, it appears that a total replacement of the plasma with either a 7% SFH solution or a 6.5% albumin solution eliminates all settling. This could be interpreted to mean that the forces which aggregate the red cells causing the settling, have been eliminated. To compare a commonly used plasma expander with SFH two samples of hydroxyethyl were used (High MW=450,000; Low MW=264,000). Both samples over a wide range of concentration greatly increase the ESR.

Table 2

The interesting feature relates to the whole blood case with different volume ratios of 6% SFH solution. These ratios are typical of those used in infusion procedures. It is observed that there is only a small change in the ESR over this clinically significant range of whole blood-SFH mixtures.

Malonamide Kinetics and Osmotic Fragility:

We have shown that the technique of hemolytic malonamide kinetics offers a means of examining the properties of the red cell membrane⁶. This method is particularly convenient and accurate for dilute solutions of SFH, that is, a 7% solution. The optics of the spectrophotometer place a limit on this

technique. In this investigation, we were concerned with the effect of overnite incubation of whole blood and 7% SFH mixtures. Previously we indicated that the kinetics could be followed by the simple mathematical expression

$$\text{Percent Hemolysis} = \frac{1}{1 + \text{EXP} [\beta(t-t_{50})]} \quad (6)$$

In equation (6), t represents the time, t_{50} is the half life of the reaction and β is a parameter measuring the breadth of the red cell kinetic activity. In (Fig.11), we Fig.11 have a sample of whole blood as the control. In (Fig.12) data Fig.12 are presented using 75% by volume of 7% SFH solution whole blood mixtures.

The values of the t_{50} are given on each figure. There does not appear to be any significant change in the hemolytic kinetics caused by the presence of the SFH solution. The data are summarized in (Table 3). Table 3

The kinetic hemolysis described above measures the ability of the red cell membrane to withstand a slowly increasing osmotic pressure (30 minute time period). It also provides information on whether or not the SFH present interferes with the transport of relatively small molecules (i.e. malonamide) across the erythrocyte membrane.

In order to measure the effect of the presence of SFH

on the red cells response to rapidly increasing osmotic pressure (1 to 2 minute time period) data were taken using a Kalmedic Fragilograph⁷. This instrument provides simultaneously cumulative and derivative hemolysis data as a function of decreasing extracellular sodium chloride concentration. These data give the mean fragility of the red cell population. The skewness of the derivative curve immediately indicates when a particular segment of the population is being preferentially attacked by a hemolytic agent. All measurements were made at 37°C using a sodium chloride-sodium veranol buffer at pH-7.4. Three experimental conditions were used. In the first instance the fragility of a mixture of 7% SFH with whole blood (50/50 by volume) was compared with a sample of the whole blood. Both of the samples were incubated at 4°C and the measurements repeated after 4 hours. Typical data can be seen in (Fig.13) Fig.13 and (Fig.14). The presence of the SFH resulted in a slight Fig.14 (7 to 8%) increase in fragility. The extra cellular saline concentration at which half the red cell population had hemolyzed, C₅₀, is listed on the graph for comparison.

The second series of experiments repeated the above protocol except that the samples were kept at room temperature (22°C) rather than 4°C. Representative curves are shown in (Figs.15 and 16). As can be seen from the C₅₀ values listed Fig.15 Fig.16

on the figure, the presence of SFH does not alter the hemolysis behavior of the erythrocytes.

Neither of the above procedures showed a significant difference in the hemolysis in the presence or absence of SFH. Similar experiments identical to those above except that the hemoglobin solution was not mixed with the whole blood until immediately before the fragility determination also showed no difference among the various samples.

The last data set compared initial fragility values with those from samples incubated for 24 hours at 37°C. An average data set is shown in (Figs. 17 and 18). It is most easily seen from (Fig.18) that initially there is no discernable difference between the whole blood and Hgb whole blood mixture. After incubating for 24 hours both samples showed an increased fragility. It should also be noted that both derivative curves are broadened but remain very symmetrical. This indicates that no given part of the population (say, only older erythrocytes) are being affected more by the treatment of the SFH. Careful attention should be given to the relative positions of the derivative curves. The SFH mixture seems to offer significant protection to the red cells during the incubation. The addition of SFH does not prevent the increase in fragility noted for the whole blood sample, but it does reduce this increased fragility. This is best seen quantitatively

Fig.1
Fig.1

by comparing the C₅₀ values listed on (Fig.17). Both of the above experimental procedures indicate that the addition of SFH does not increase the susceptibility of the red cell to osmotic fragility and may offer a slight but significant degree of protection.

Fig.17

Some New Directions:

Since one of the major concerns with the clinical use of SFH is its relatively short dwell time, two approaches to this problem are currently being investigated. The first deals with mixtures of hydroxyethyl starch (HES) and SFH and the second approach involves the formation of an SFH polymer complex.

In (Fig.19) the oncotic pressures of low molecular weight HES ($M_w=264,000$), high molecular weight HES ($M_w=450,000$) and SFH are compared. There are differences but they are not of major significance. However, if the relative viscosities are examined, as shown in (Fig.20), notable differences are readily apparent. It is possible to lower the viscosity of the HES by the addition of SFH. This is illustrated in (Fig.21) for both of the HESes. The osmotic pressure was determined on the mixtures for the high molecular weight HES with SFH. The effect is not as dramatic but this is related to the similarities in the osmotic pressures of the pure materials.

Fig.19

Fig.20

Fig.21

However, detailed in vivo testing will be necessary to substantiate the usefulness of the mixtures (Fig.22).

Fig.22

Current work in our laboratory has indicated that it is possible to react Hgb with water-soluble polymers⁸. Two of these compounds have been synthesized. The first is shown on (Fig.23), between HES and Hgb. The characterization of this polymer indicates a number average molecular weight of about 275,000. This indicates that about 3 Hgb molecules have reacted with each HES molecule of number average molecular weight 65,200. However at a 3 gm/100ml concentration level, the relative viscosity is approximately 2.1 times the viscosity of SFH and therefore may present a problem. If a fractionated HES sample is used with a lower molecular weight, there will be better control of both the viscous and oncotic properties.

Fig.23

Another approach to synthesizing a water soluble polymer with oxygen carrying properties has been to use a Tetronic polyol as a starting material. These compounds are four-pronged polymers of ethylene and propylene oxides. They are shown in (Fig.24). It is possible to convert the alcohol groups to a tetra aldehyde under very mild conditions⁹. By regulating the reaction conditions, it appears that it is possible to obtain nono-, di-, tri-and tetra-substitution. Our current interest concerns the separation of these mixtures.

Fig.24

Conclusions:

The preliminary results of this study indicate that the biophysical characteristics of SFH make it an extremely suitable blood substitute. One of the key factors in this conclusion is its availability from out-dated whole blood. At the concentration that is currently being considered as an infusing solution, 7%, it is a Newtonian fluid having a viscosity very similar to plasma. At this concentration, SFH also has very favorable hemo-dilution properties. This is a feature which is a critical factor for any plasma substitute. However, the added advantage here is the potential oxygen transport ability which most expanders lack. There also appears to be little interaction between the plasma proteins and SFH in the concentration range between 7 and 14% as indicated from viscosity measurements. However, at concentrations exceeding 10% SFH solutions exhibit a slight non-Newtonian flow pattern that may present some complications in the microcirculation and distract from its advantage as a hemo-diluent.

The osmotic behavior of SFH solutions indicate that it approaches an ideal polymer-solvent system as illustrated by the small value of the second virial coefficient, especially when compared to other plasma substitutes including dextran, hydroxyethyl starch and polyvinyl pyrrolidone. In mixtures with whole blood at a series of hematocrits, SFH solutions at

concentration between 7 and 15.2% all appear to be hyperosmotic. This biophysical property could certainly have significant ramifications in cases of severe shock.

Another notable feature of SFH solutions is its ability to maintain the integrity of whole blood and not to initiate aggregation of the erythrocytes. A common technique which illustrates this phenomena is the ESR. With some plasma expanders, such as dextran and polyvinyl pyrrolidone, there is a noticeable increased ESR. Our studies with HES, both the high and low molecular weight types, also indicate an increased ESR. The investigations of the ESR in this study with SFH, either alone with whole blood or in combination with the low molecular weight HES, indicate the potential usefulness of SFH in maintaining stability in these systems. The fact that SFH does not promote erythrocyte aggregation is another advantage that it may have as an infusion fluid for the treatment of shock.

In the presence of SFH solutions, the transport properties of the red cell membrane are apparently not effectively altered. This has been illustrated in the hemolytic malonamide kinetic studies. The negligible changes in the half life values for this kinetic process over a twenty-four hour period substantiate this fact. To further explore the erythrocyte membrane properties similar studies of the osmotic fragility were performed.

Although the results are preliminary, it should be stressed that SFH solutions may offer protection against hemolysis when used as a transfusion solution.

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REFERENCES

- 1) Mooney, M: The viscosity of a concentrated suspension of spherical particles. J. Colloid Sci. 6: 162, 1951
- 2) Simha, R: The influence of Brownian movement on the viscosity of the solution. J. Phys. Chem. 44: 25, 1940
- 3) Tanford, C: Physical Chemistry of Macromolecules
New York, John Wiley and Sons, Inc. 1967
- 4) Chien, S et.al.: Aspects os sickle cell disease
in Proc. Symp. Mol. Cell., DHEW Pub. Na(NIH) 76-1007,
p.277, 1976
- 5) Misiaszek, E; Williams, R; Stasiw, D; Cerny, L: An
automatic sedimentimeter. Biorheology 14: 145, 1977
- 6) DeTraglia, M; Cook, FB; Stasiw, D; Cerny, L: Kinetics
of malonamide induced hemolysis of human erythrocytes
an autocatalytic effect. Bio. Chim. Biophys. Acta
345: 305, 1974
- 7) Stasiw, D; Rosato, S; Mazza, J; Cerny, L: Quantitative
osmotic fragility and disease states: A preliminary
study. J. Lab. Clin. Invest. 89: 409, 1977
- 8) Baldwin, JE: Private communication
- 9) Baldwin, JE; Whitten, J: Private communication

Table 1

The Osmotic Pressure and Intrinsic Viscosity of Stroma-Free Hemoglobin and Some Plasma Substitutes

<u>Substance</u>	<u>M_n</u>	<u>r₂</u> ($\frac{\text{ml}}{\text{gm}}$)	<u>[η]</u> ($\frac{\text{dl}}{\text{gm}}$)	<u>r₂/[η]</u>
Temperature 37°C				
SFH	69,200	3.59	0.0385	93.2
Albumin	81,000	7.01	0.0380	185.0
Temperature 30°C				
SFH	67,300	3.49	0.0350	99.7
Dextran- Rheomacrodex	33,400	12.4	0.175	70.9
Dextran- Macrodex	49,100	20.2	0.275	73.3
PVP-Plasdone	23,700	8.68	0.202	43.0
Hydroxyethyl- Starch	81,500	21.2	0.181	117
Temperature 20°C				
SFH	66,800	3.21	0.0320	100.3

The Effects of Replacement Fluids on the Erythrocyte Sedimentation Rate

<u>System</u>		<u>Hct</u>	<u>Vmax</u> ($\frac{cm}{hr}$)
A	1) Whole Blood	41	3.67
	2) Washed cells in 7% SFH	41	no settling
B	1) Whole Blood	36	7.98
	2) Washed cells in 50% plasma+50% 7% SFH	36	1.77
	3) Washed cells in 50% plasma+50% 0.9% Saline	36	1.01
C	1) Whole Blood	42	11.08
	2) Washed cells in 66% plasma-34% 10% SFH	42	4.62
D	1) Whole Blood	42.5	4.69
	2) Washed cells in 6.5% Albumin	42.5	no settling
E Washed Cells in			
	1) 1% High MW HES	38	1.01
	2) 2% High MW HES	38	6.46
	3) 3% High MW HES	38	18.37
	4) 4% High MW HES	38	26.98
F Washed Cells in			
	1) 2% Low MW HES	37	0.63
	2) 4% Low MW HES	41	6.21
	3) 6% Low MW HES	38	10.39
G Washed Cells in			
	1) 6% Low MW HES+0% SFH	36	11.15
	2) 6% Low MW HES+0.606% SFH	36.5	5.32
	3) 6% Low MW HES+0.303% SFH	37	8.11
H Whole Blood			
	1) 6% SFH-Whole Blood (lv:6v)	38	6.78
	2) 6% SFH-Whole Blood (lv:7v)	31.5	5.95
	3) 6% SFH-Whole Blood (lv:9v)	32	5.95
		34	5.95

Table 3
 Malonamide Induced Hemolytic Kinetics
 Effect of Stroma Free Hemoglobin Concentration
 and Volume Percent Whole Blood

SFH Conc.	Control Blood Sample	Overnight	75%		Overnight	50%		Overnight	25%		Overnight
			Control	25% SFH		Control	50% SFH		Control	75% SFH	
3.17%	S	0.591	0.485	0.437	0.457	0.539	0.403	0.475	0.423		
	t50	15.5	17.0	13.9	14.8	12.9	15.4	12.1	12.9		
7.00%	S	0.271	0.276	0.215	0.222	0.256	0.232	0.239	0.261		
	t50	20.7	18.3	16.6	14.9	17.4	15.9	17.7	18.2		
9.5%	S	0.407	0.359	0.259	0.395	0.328	0.383	0.384	0.386		
	t50	15.8	14.8	15.2	16.3	14.7	16.2	16.3	16.7		
	S	0.358	0.333	0.451	0.443	0.336	0.284	—	0.195		
	t50	19.4	21.1	17.2	16.7	16.9	18.2	—	19.5		

$$\text{Percent Hemolysis} = \frac{1}{1 + \exp [\beta(t - t_{50})]}$$

β in min^{-1}

t_{50} in min

Figure 1:

The Relative Viscosity of Hemoglobin
and Albumin Solutions

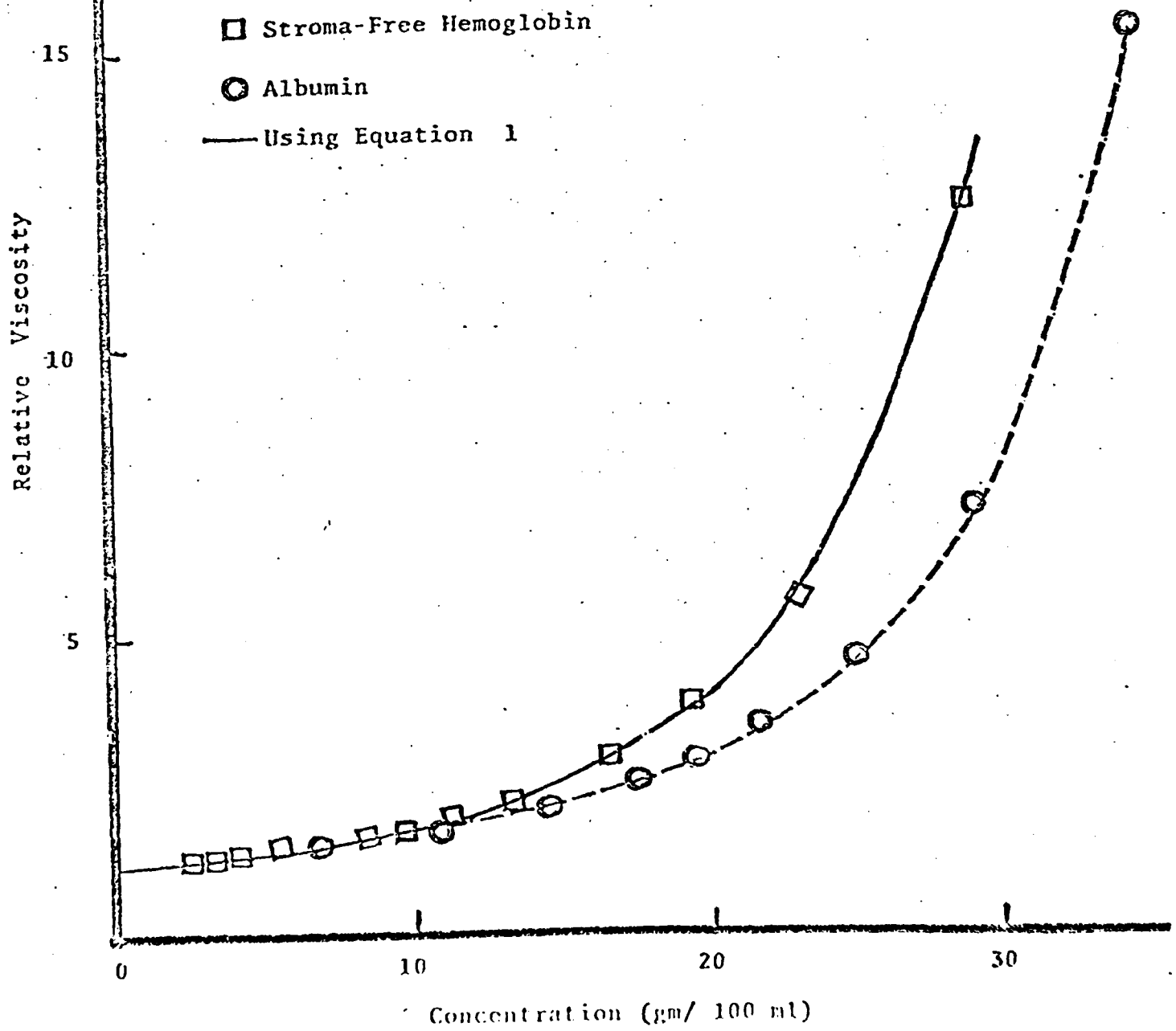
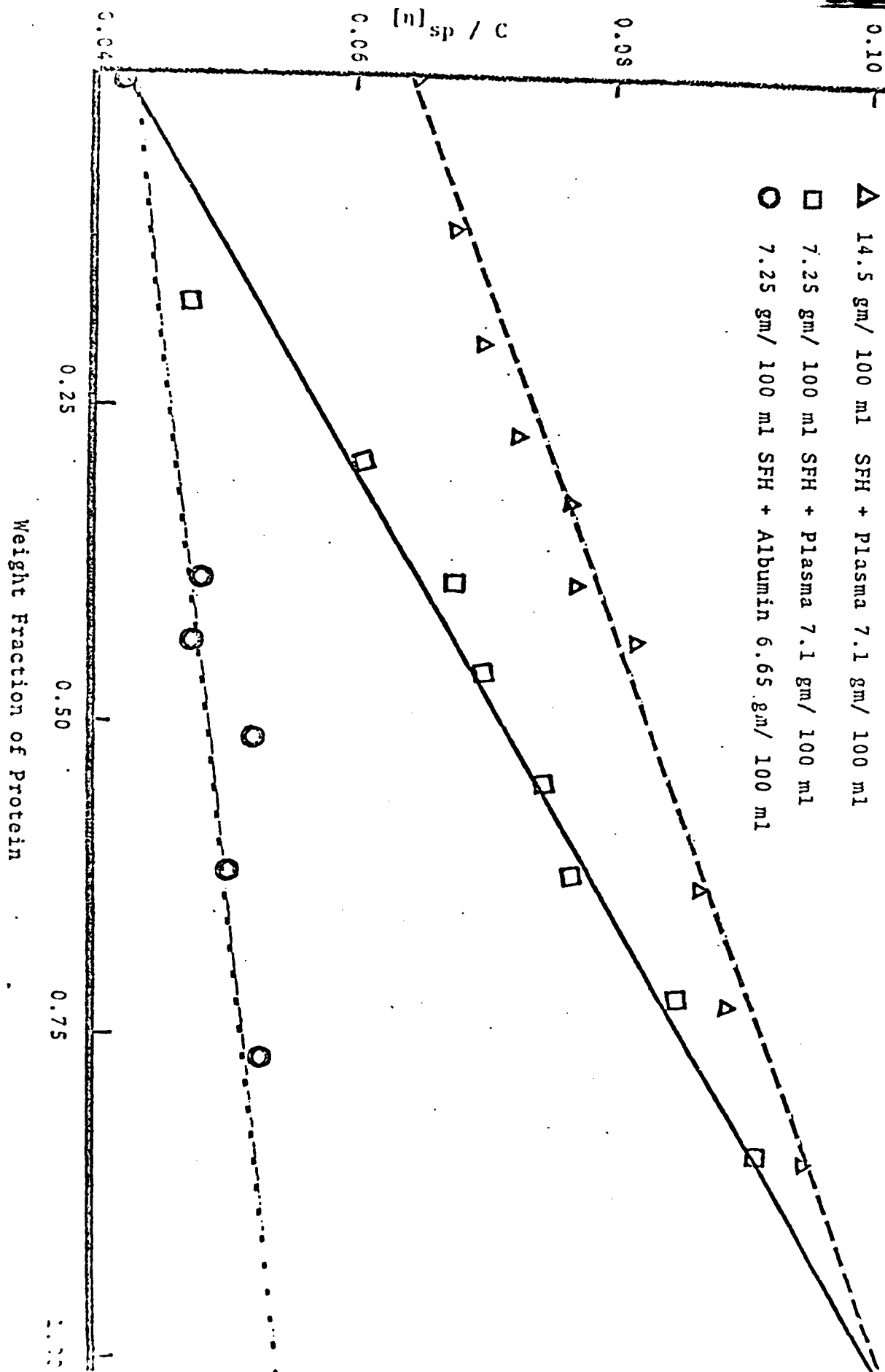


FIGURE 2 :

The Viscosity of Mixtures of Hemoglobin and Plasma Proteins



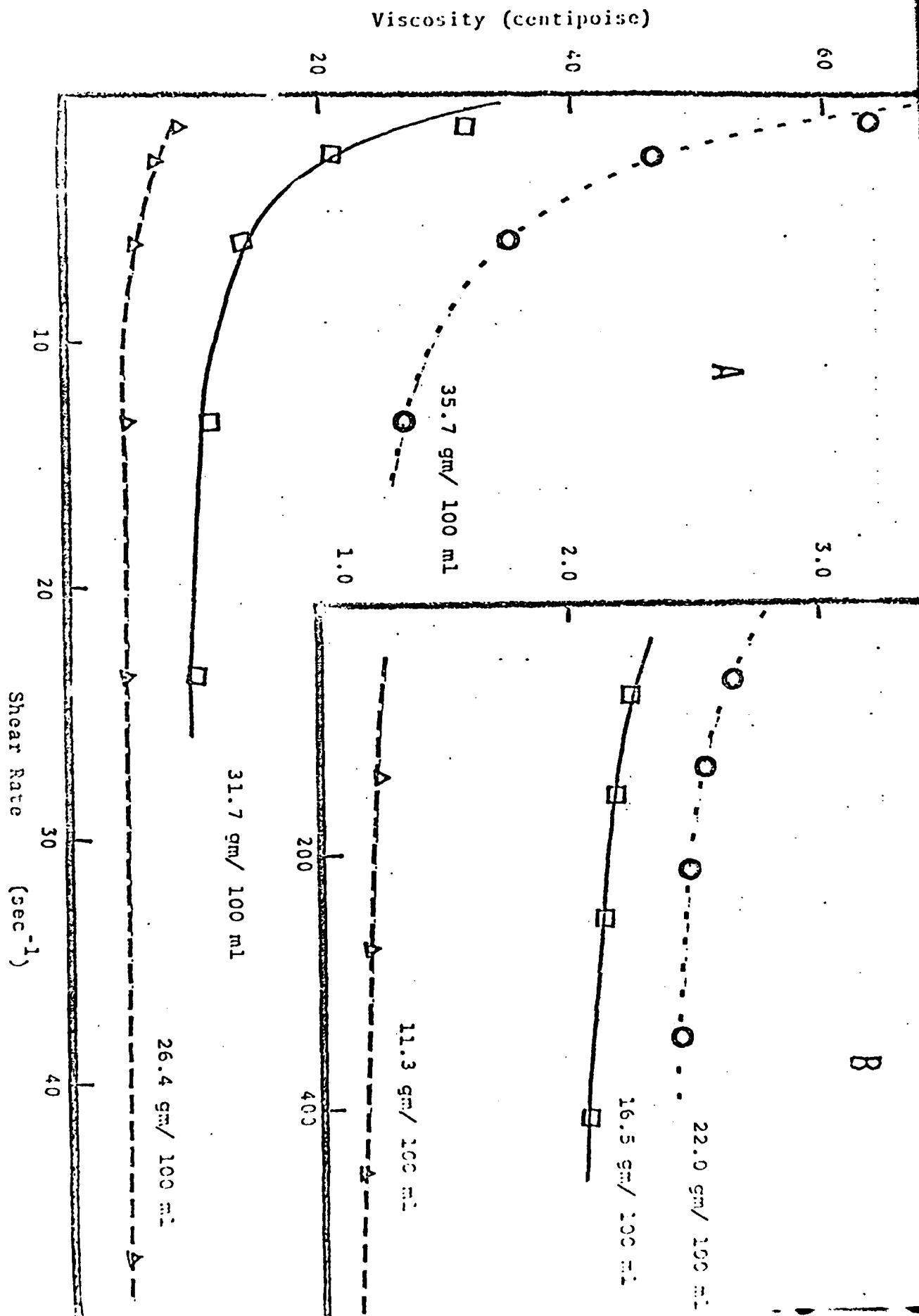
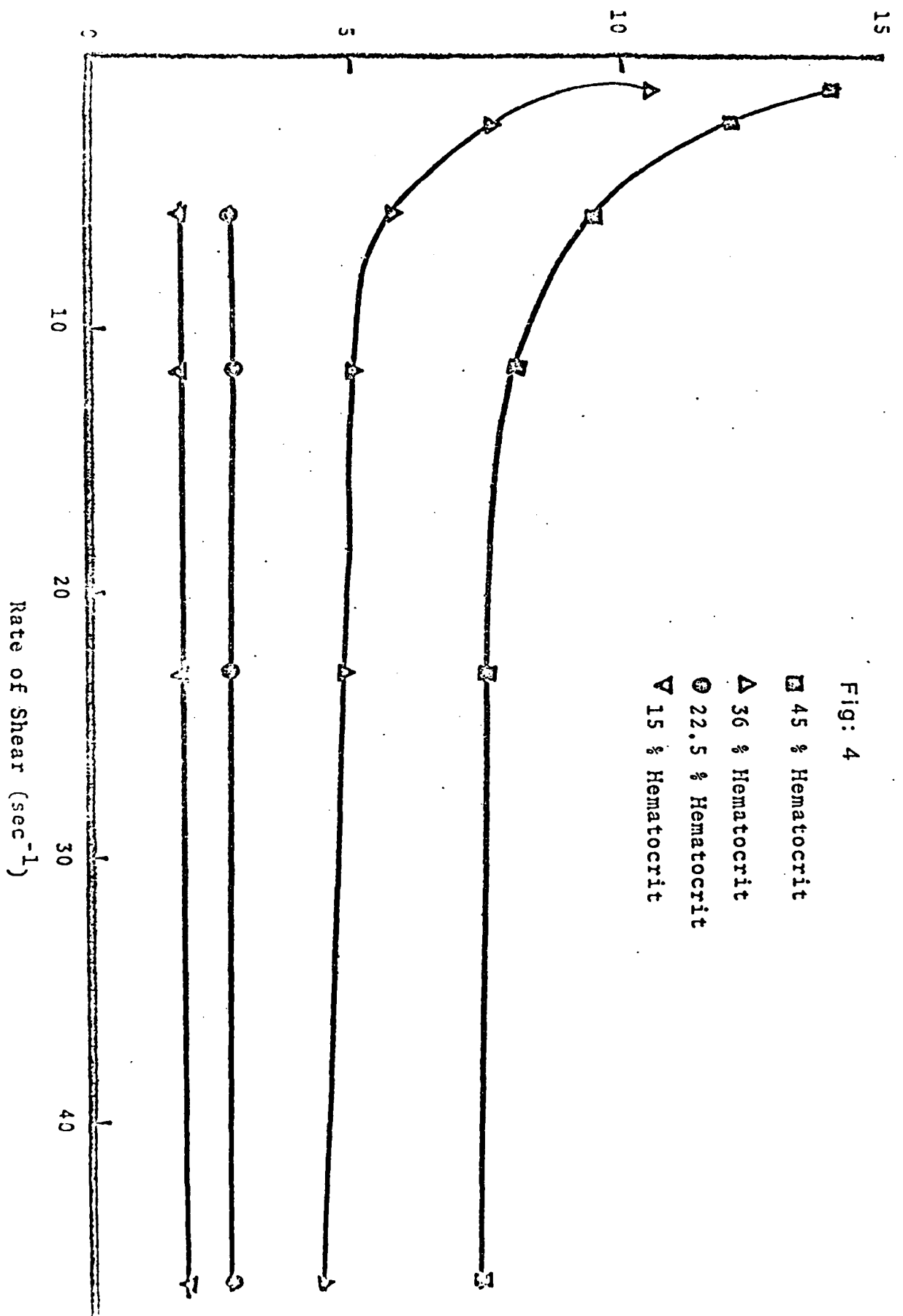


FIG 3: The Effect of Shear Rate on the Viscosity of Hemoglobin Solutions



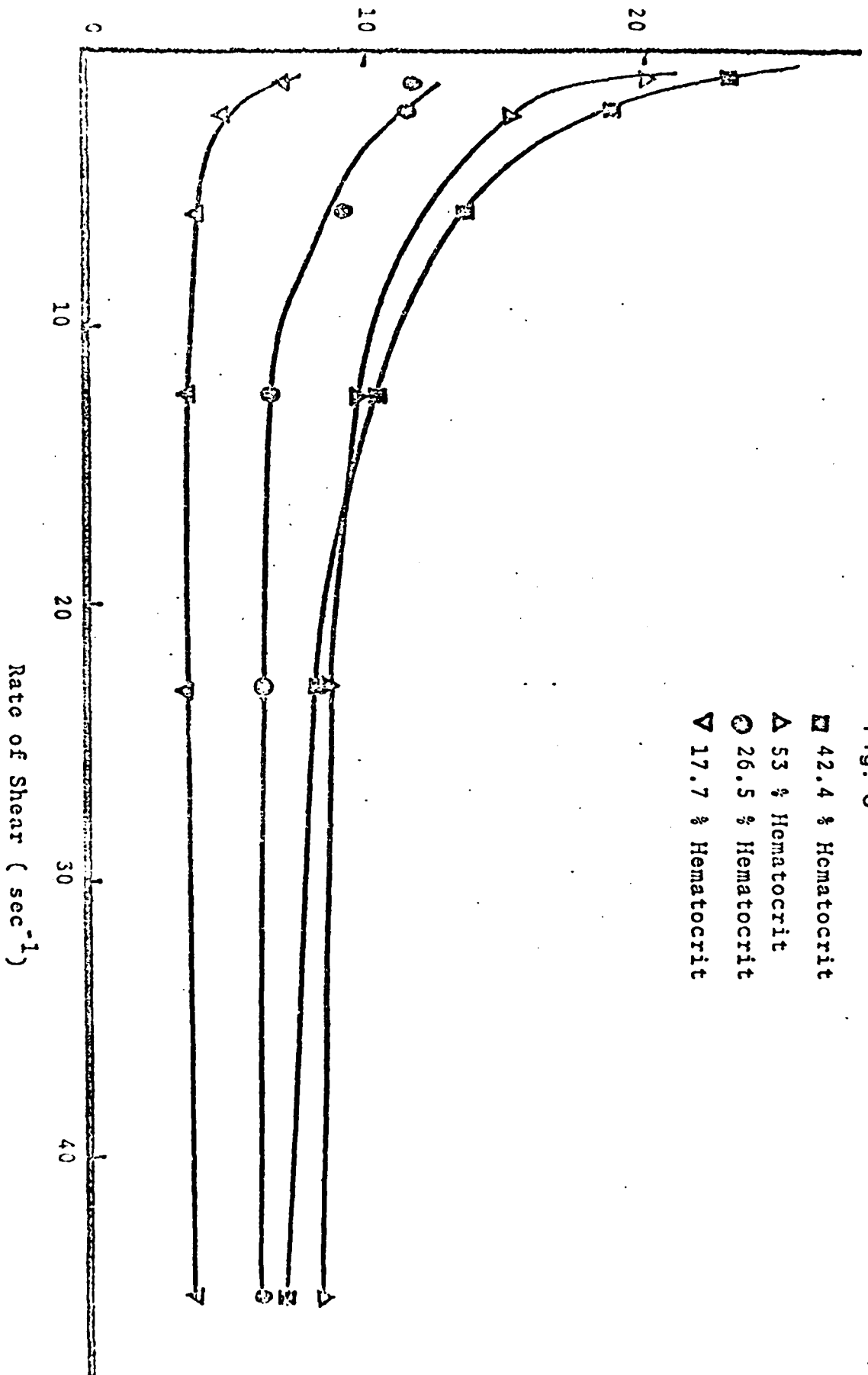
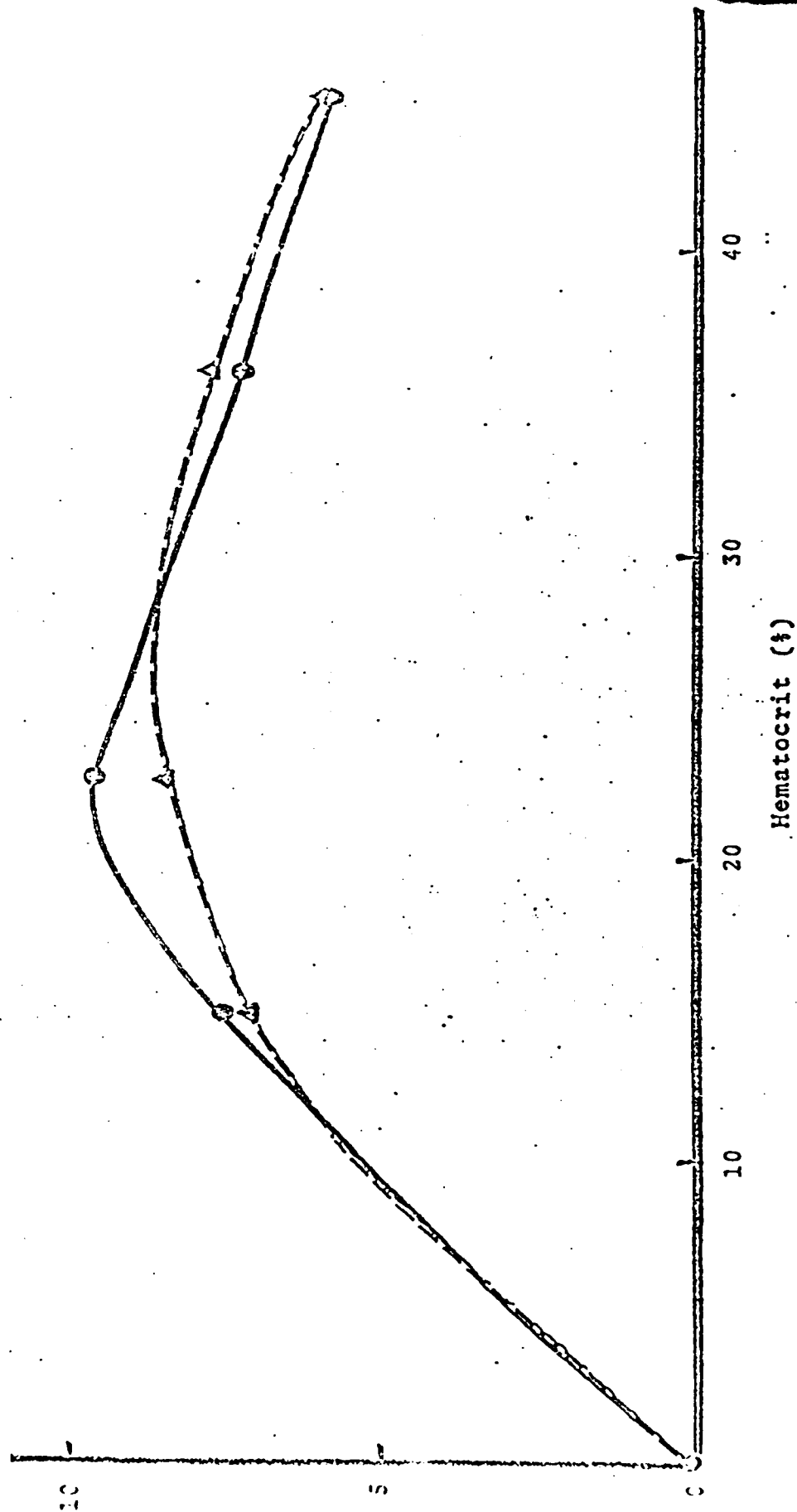
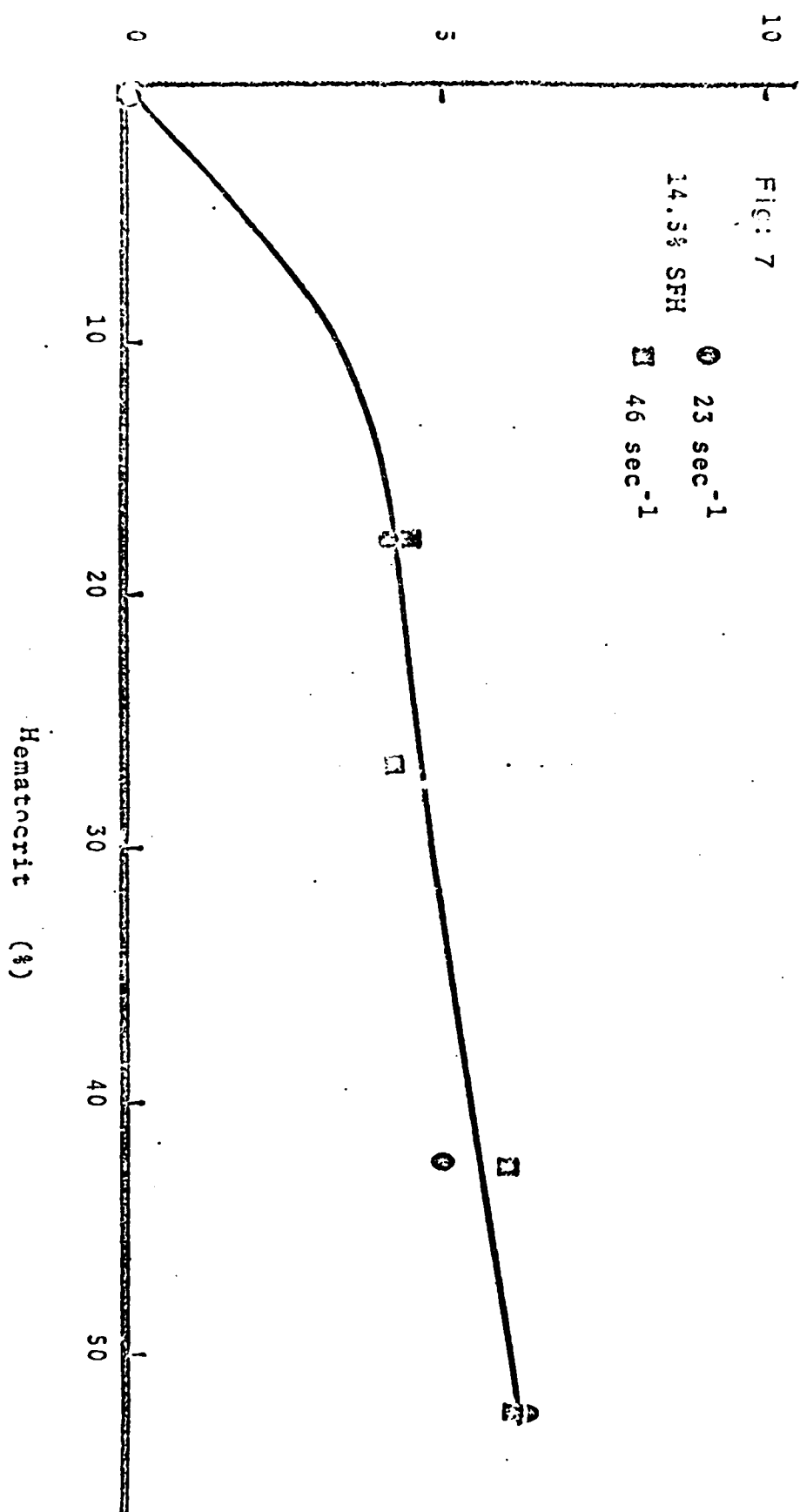


FIG. 6 : The Viscosity and Apparent Oxygen Transport in 6.1% SFH

Δ 46 sec⁻¹
 $\circ$ 23 sec⁻¹





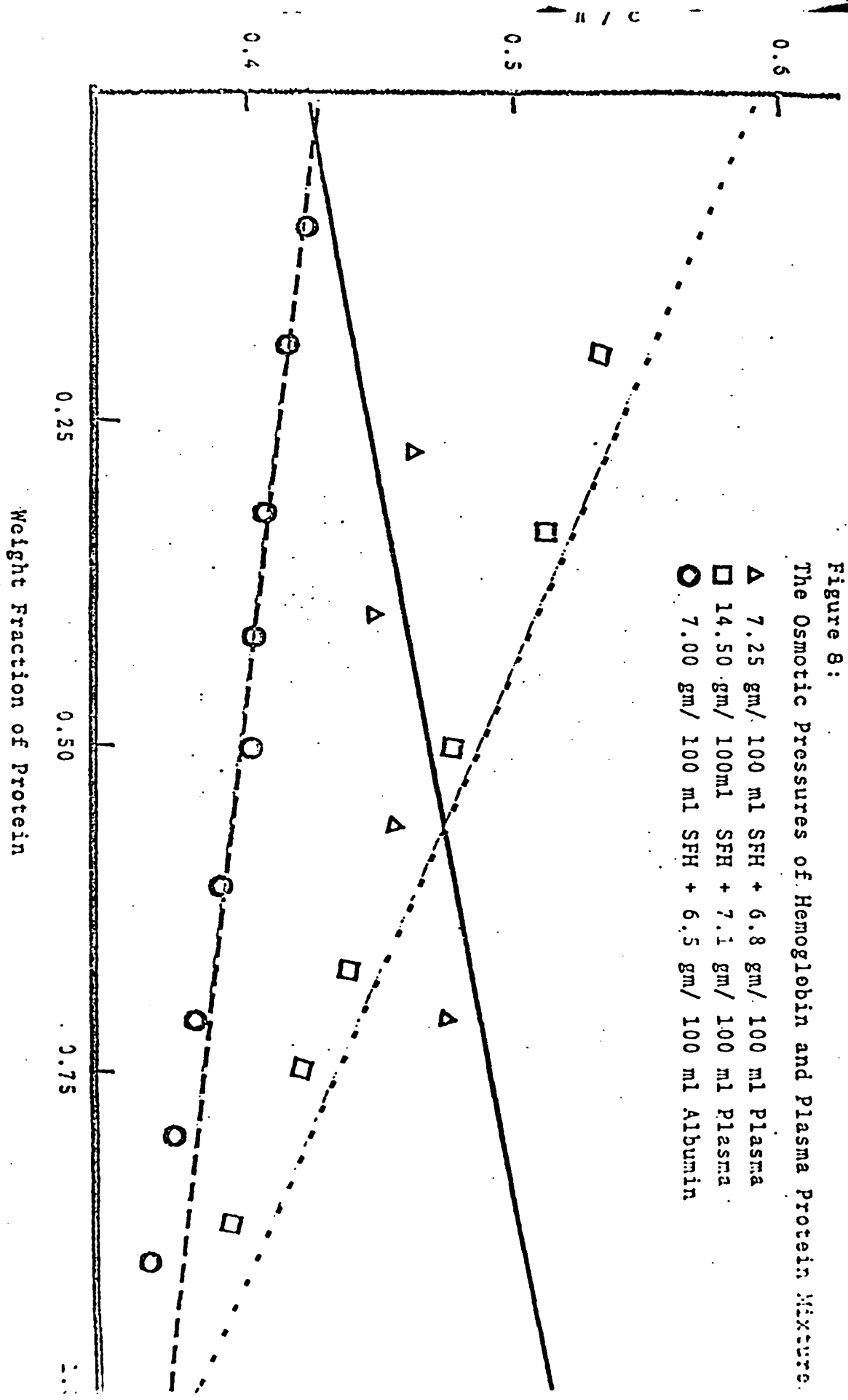
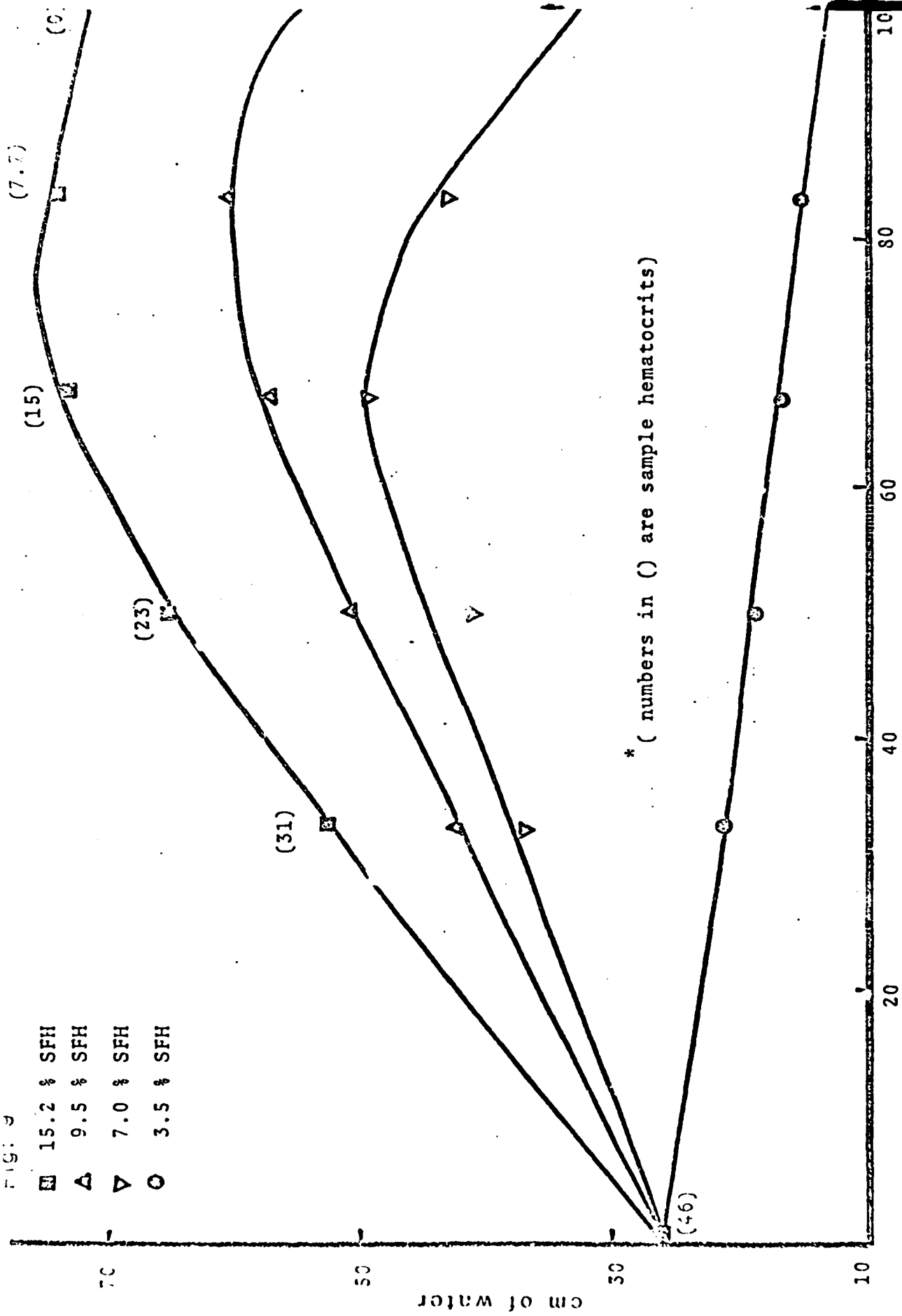


FIG. 2

■ 15.2 % SFH
 ▲ 9.5 % SFH
 ▼ 7.0 % SFH
 ○ 3.5 % SFH



* (numbers in () are sample hematocrits)

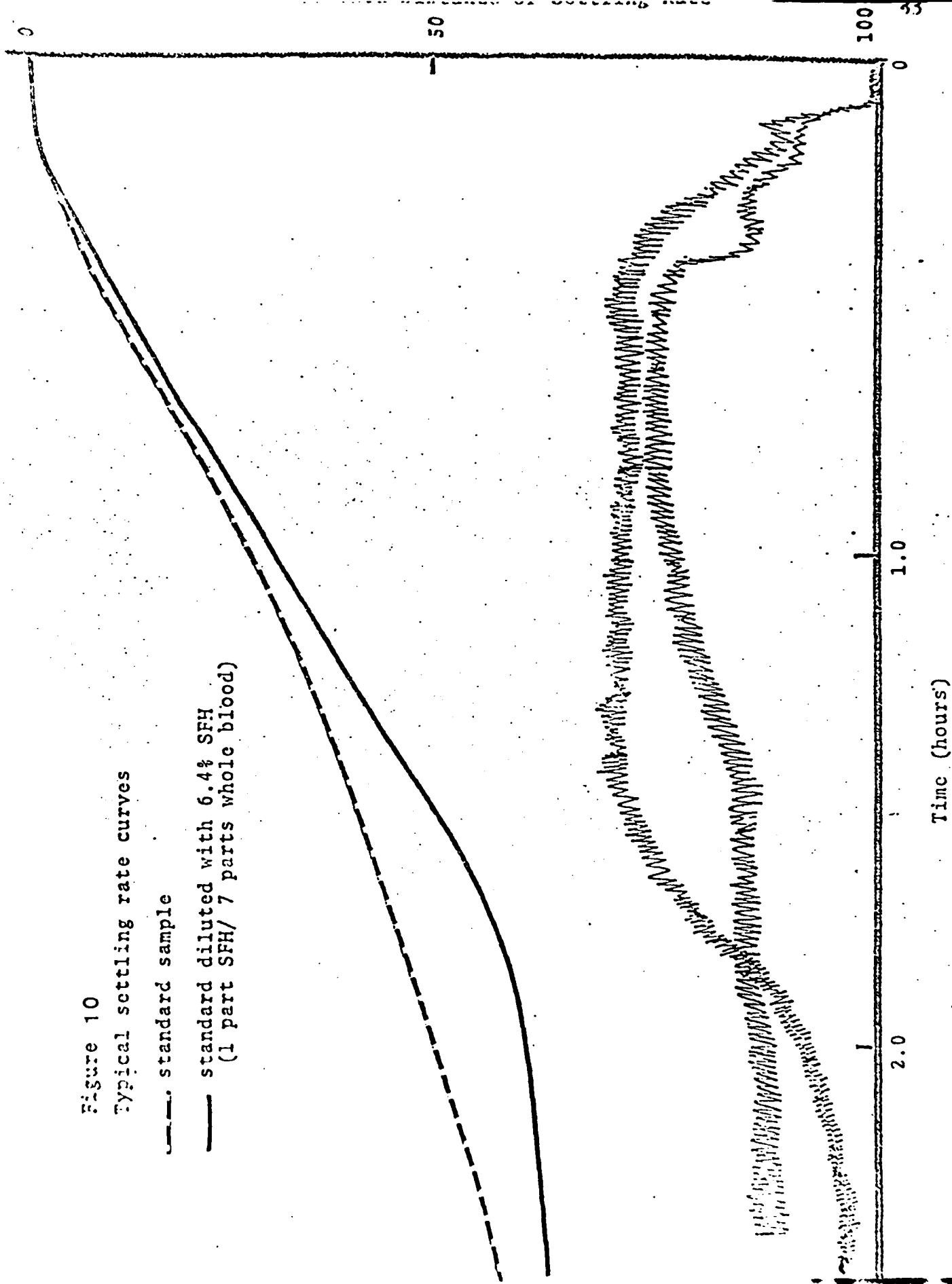
Volume Percent SFH

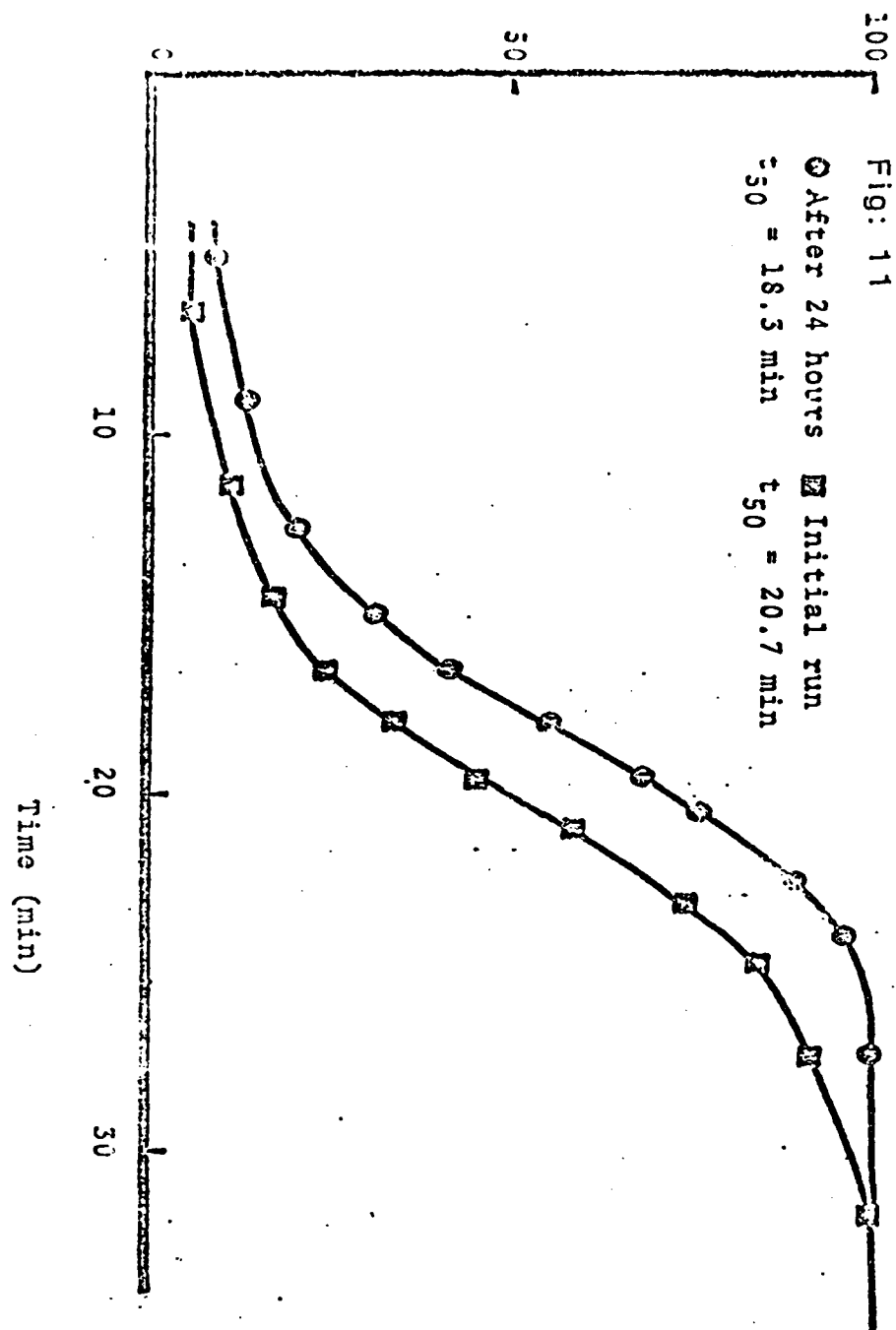
Figure 10

Typical settling rate curves

--- standard sample

— standard diluted with 6.4% SFH
(1 part SFH/ 7 parts whole blood)





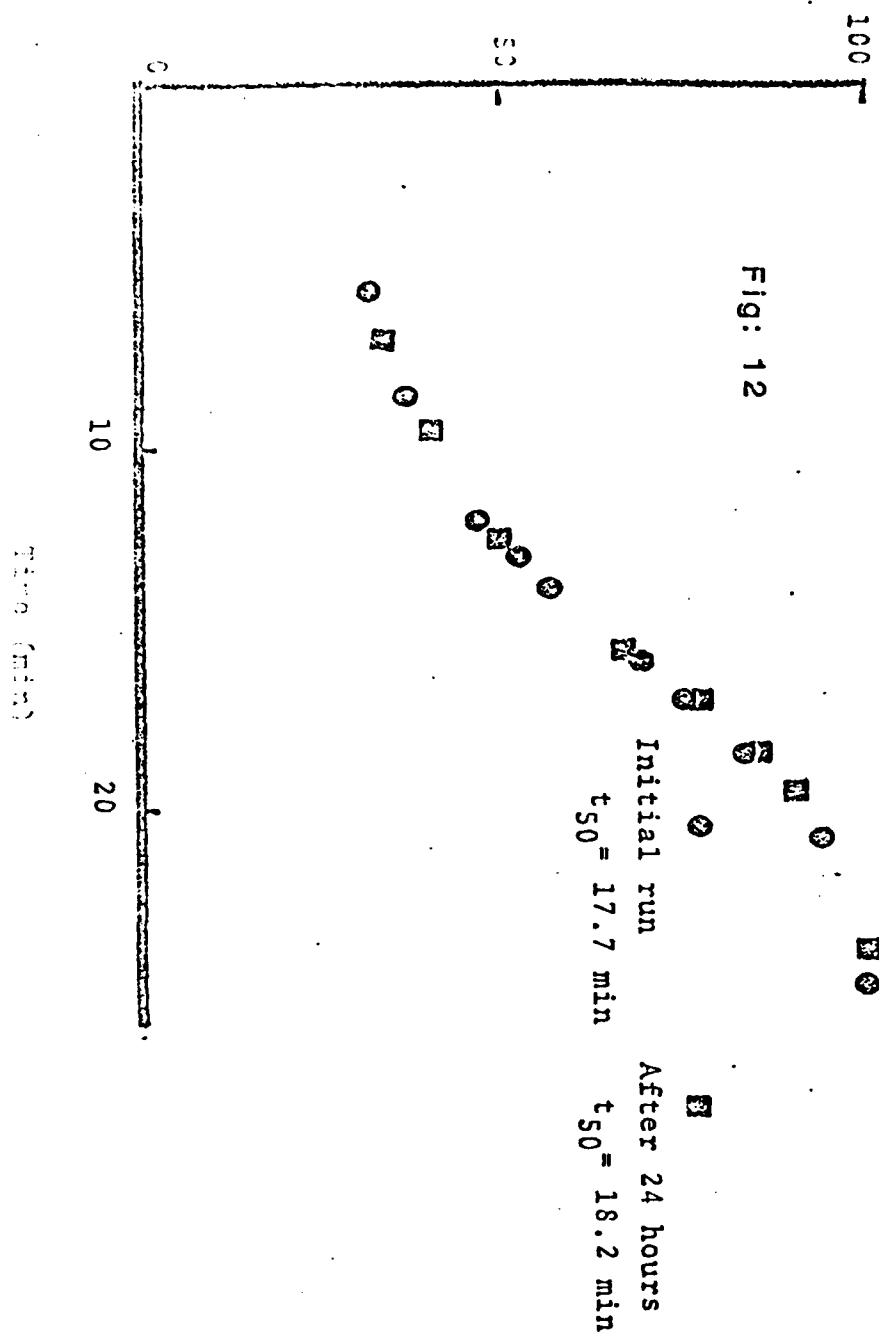


FIG: 13

C₅₀ Values

Whole blood Hgb-Whole blood Mix

Initial: 0.37g/l Initial: 0.44 g/l

After 4 hours: 0.38g/l After: 0.44 g/l

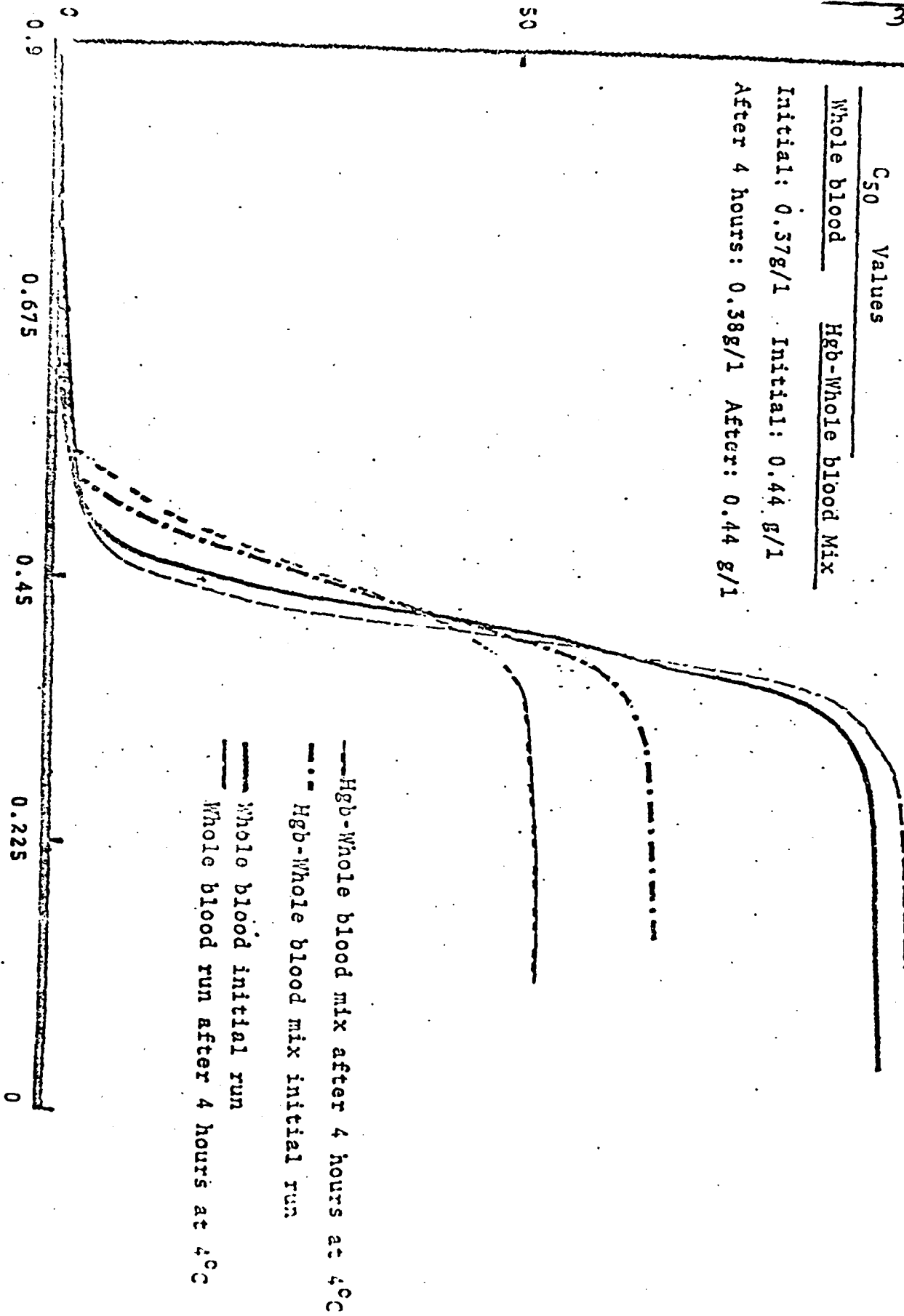
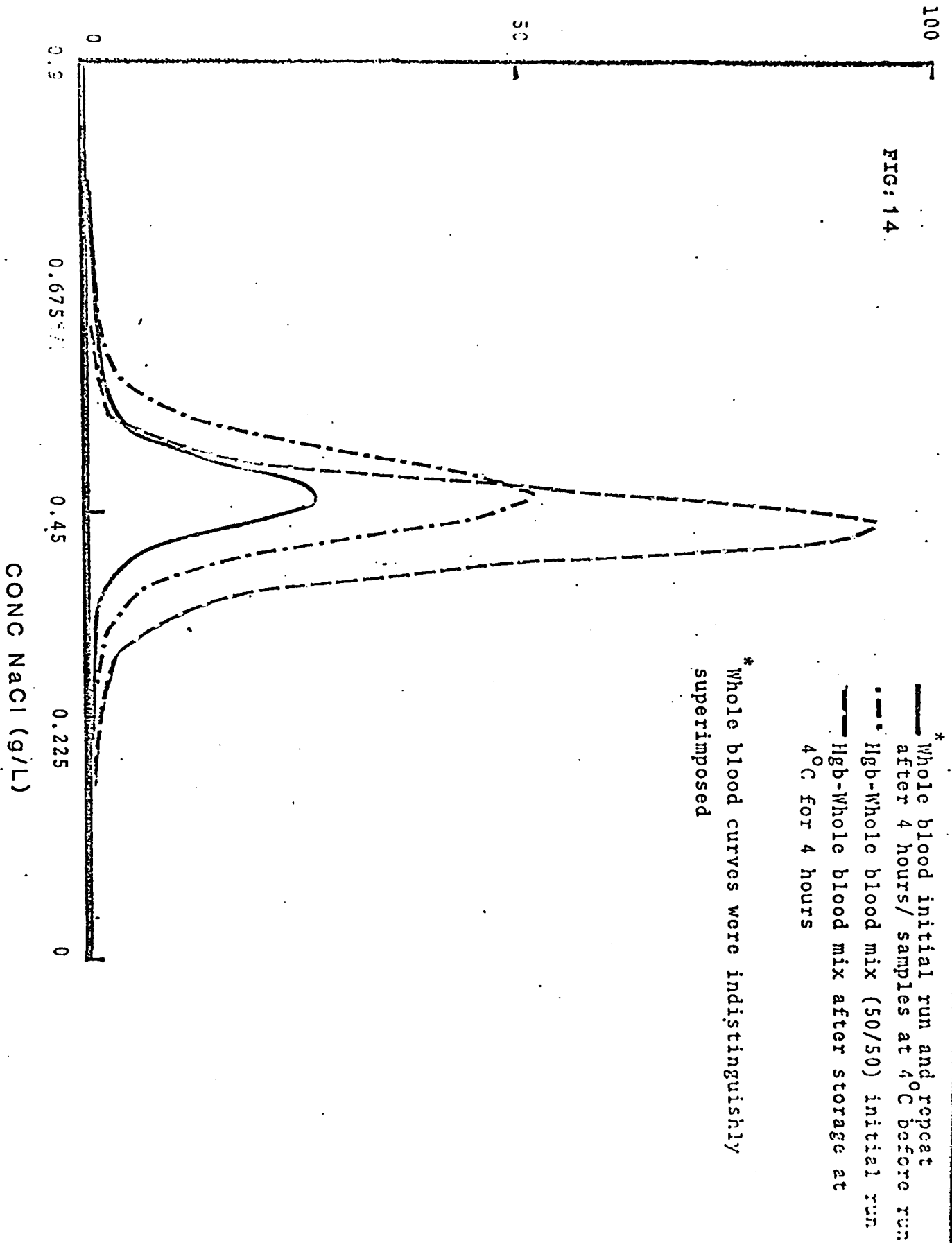


FIG: 14



C₅₀ Values

Whole blood

Hgb-Whole blood Mix

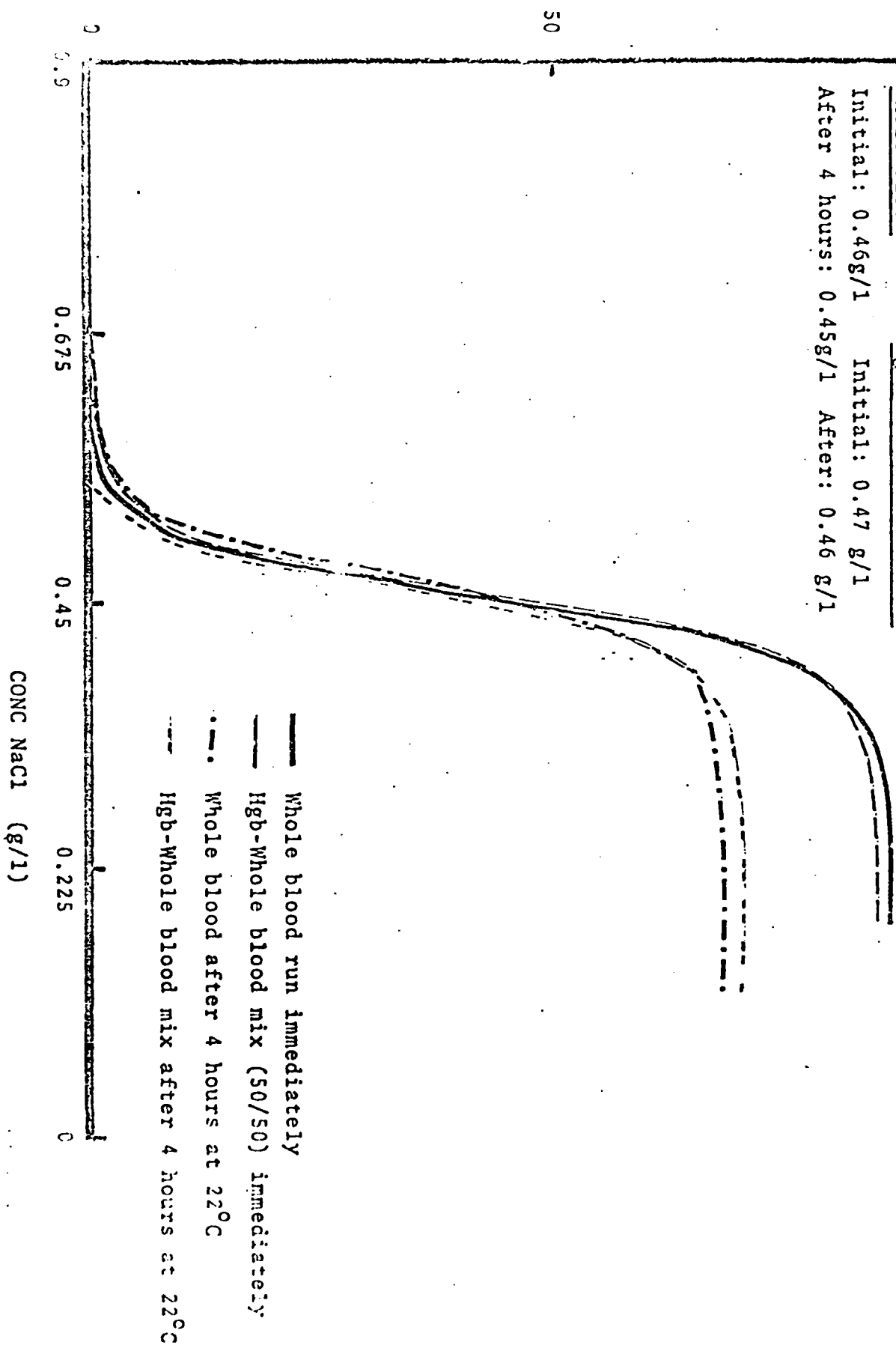
Initial: 0.46g/l

Initial: 0.47 g/l

After 4 hours: 0.45g/l

After: 0.46 g/l

Fig: 15

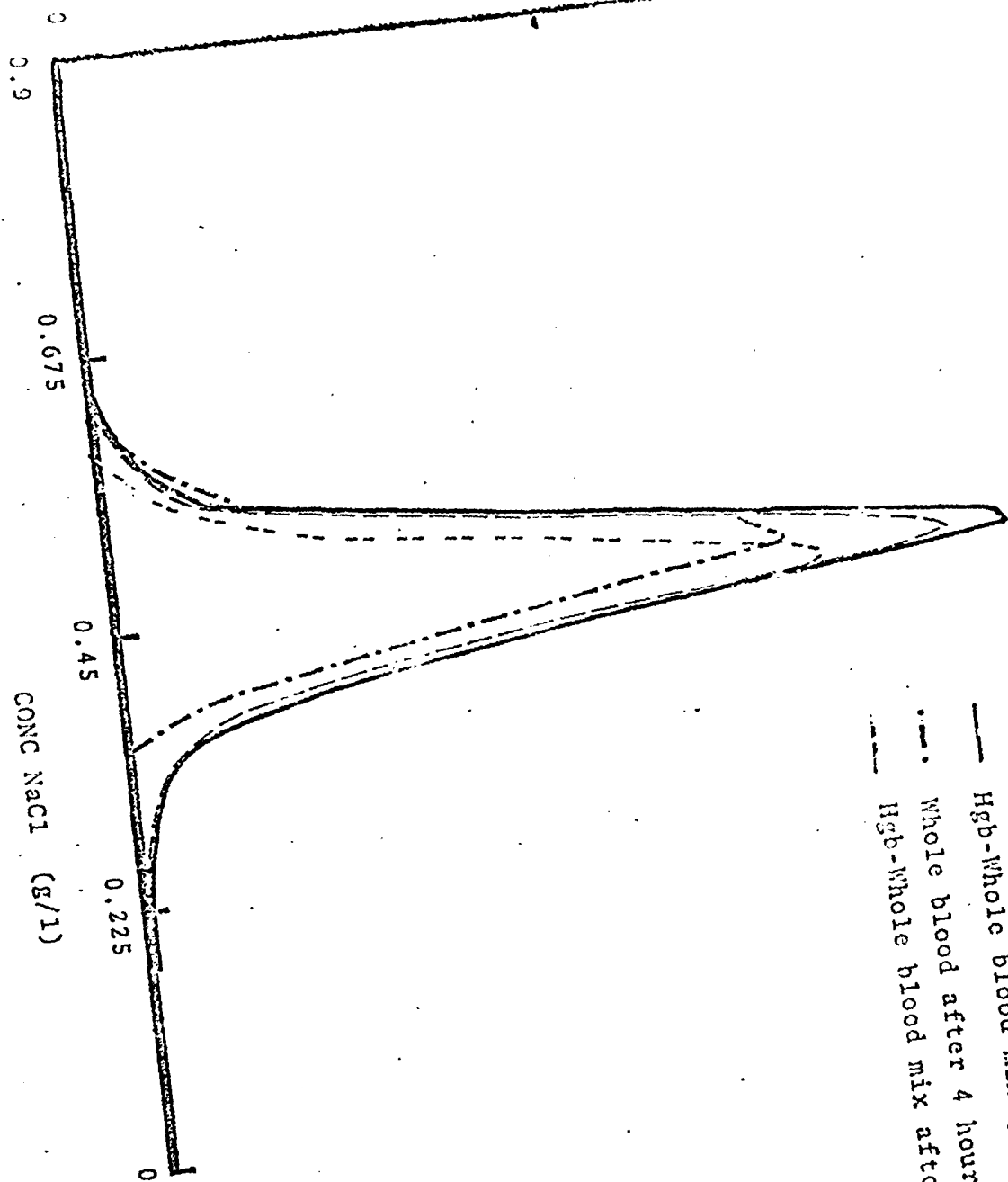


Percent Relative Change in Transmittance

100

50

FIG: 16



- Whole blood run immediately
- - - Hgb-Whole blood mix (50/50) immediately
- · - Whole blood after 4 hours at 22°C
- - - Hgb-Whole blood mix after 4 hours at 22°C

FIG: 17

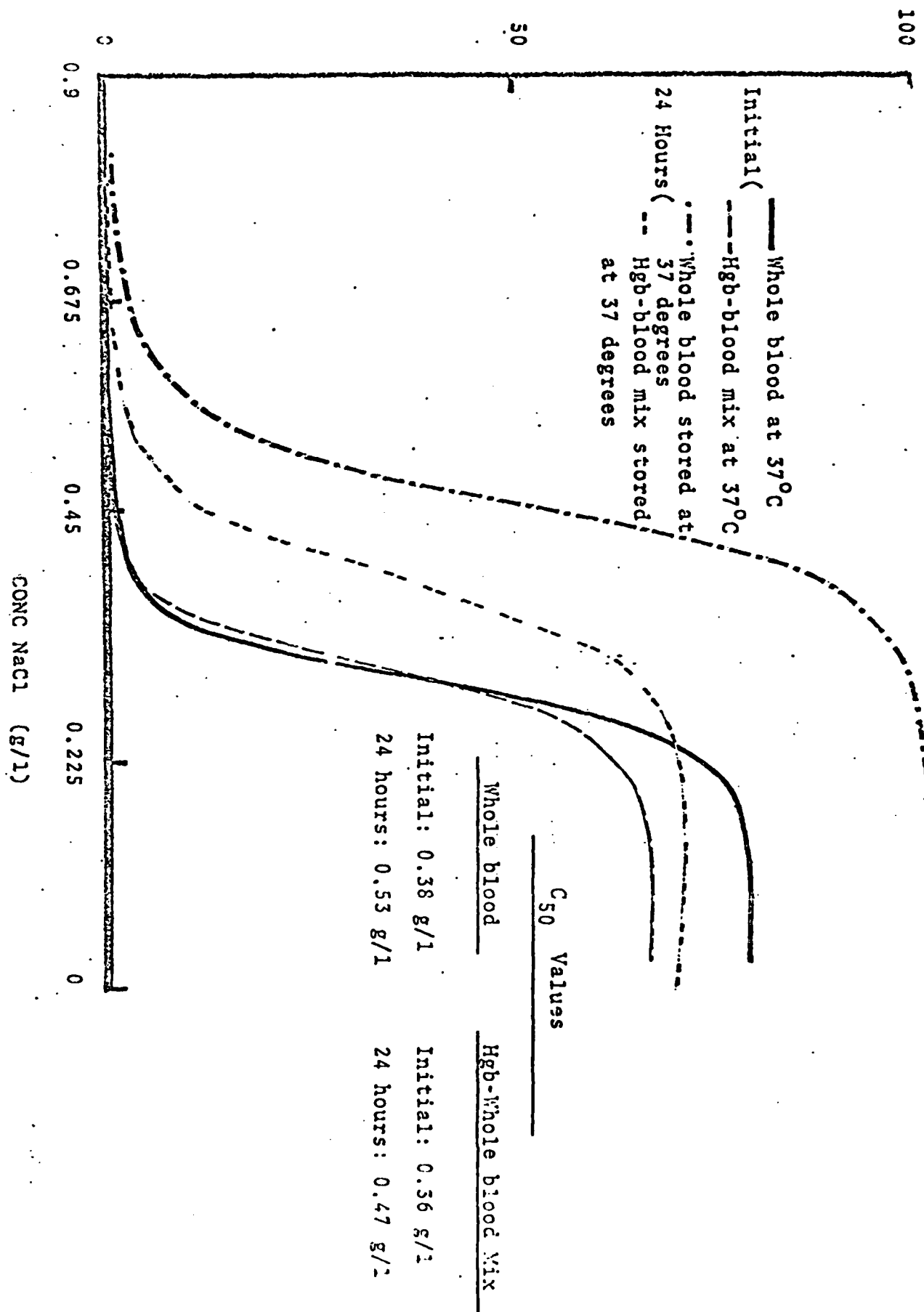
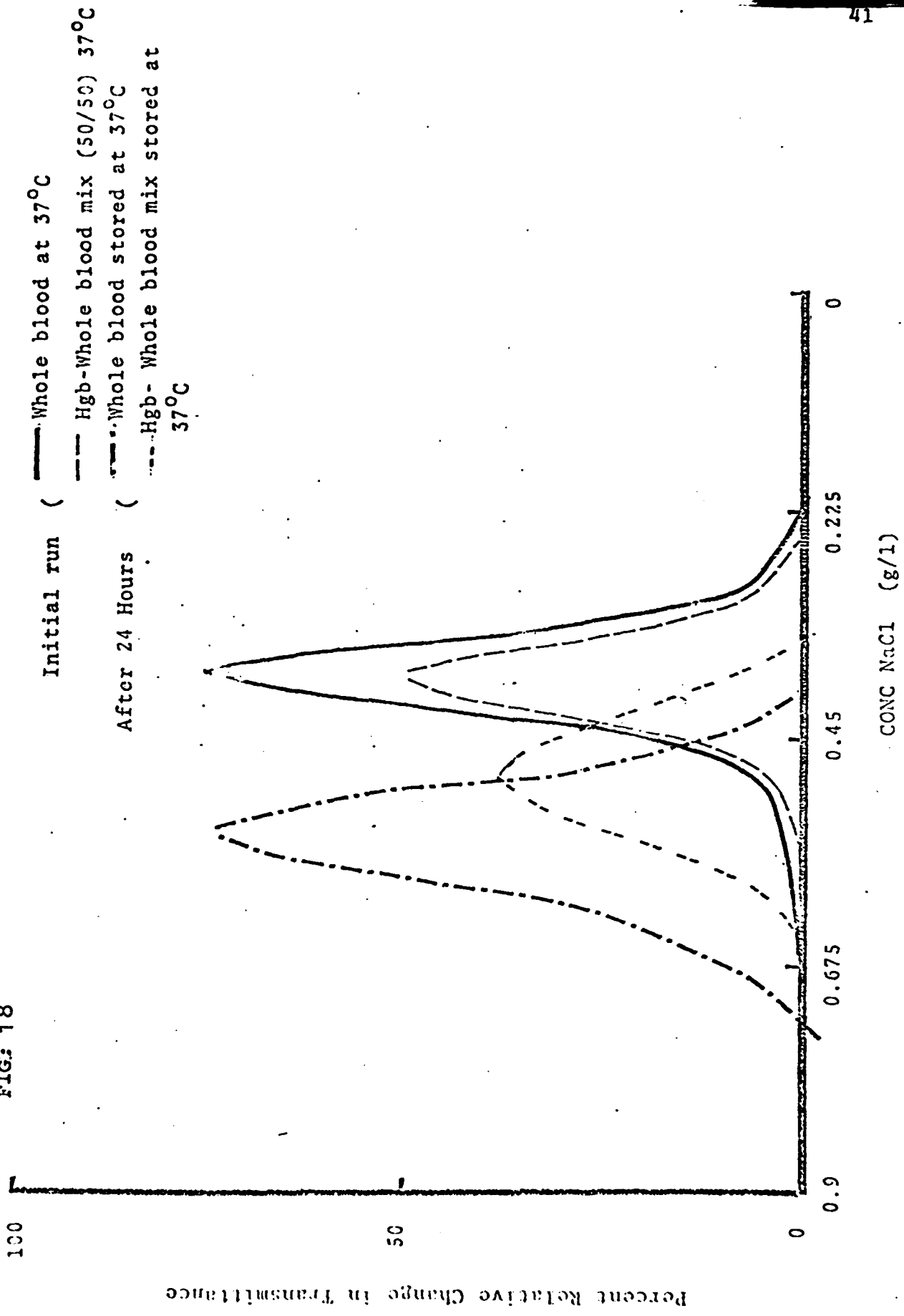
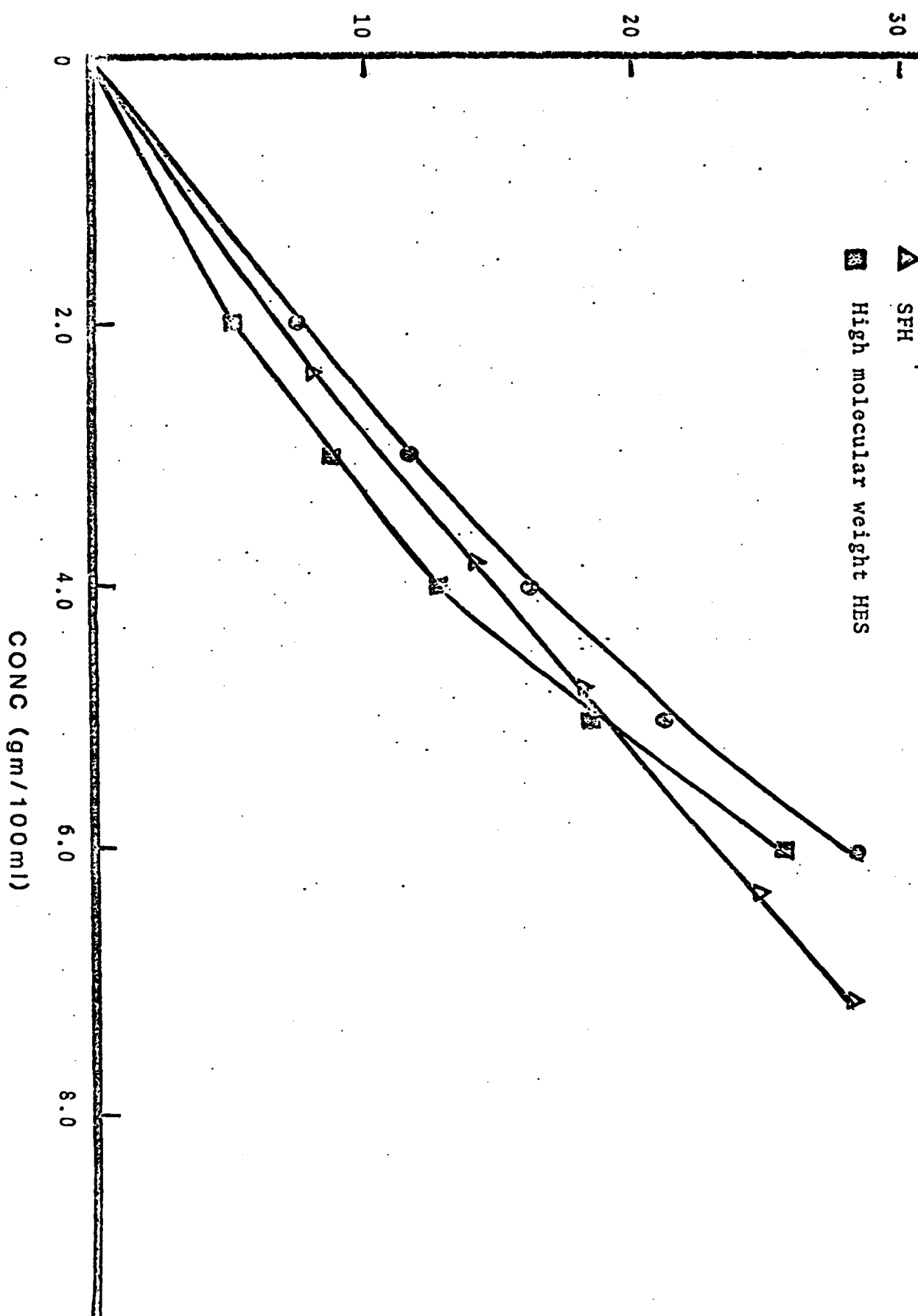


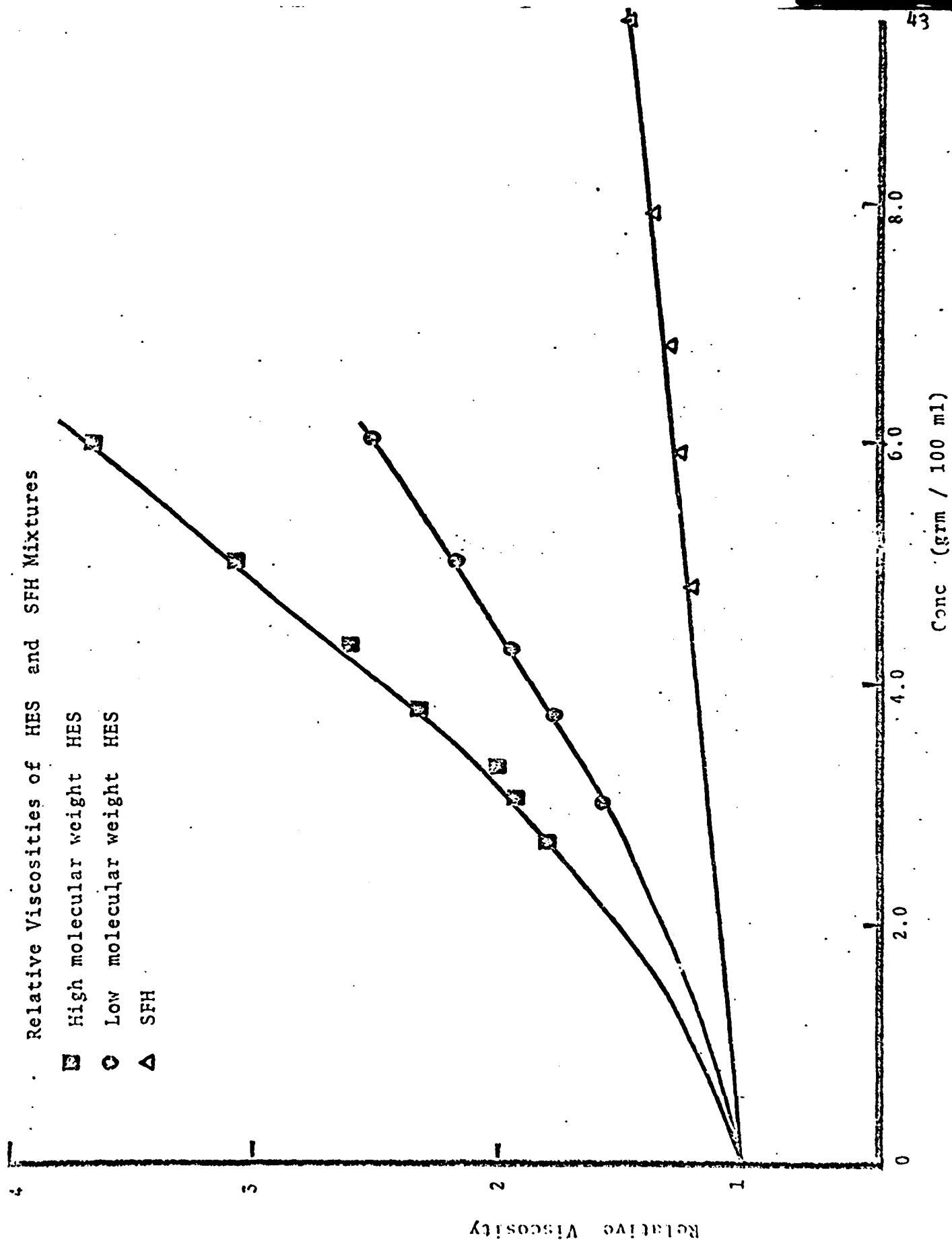
FIG: 18



h (cm of H_2O)

Relative Viscosities of HES and SFH Mixtures

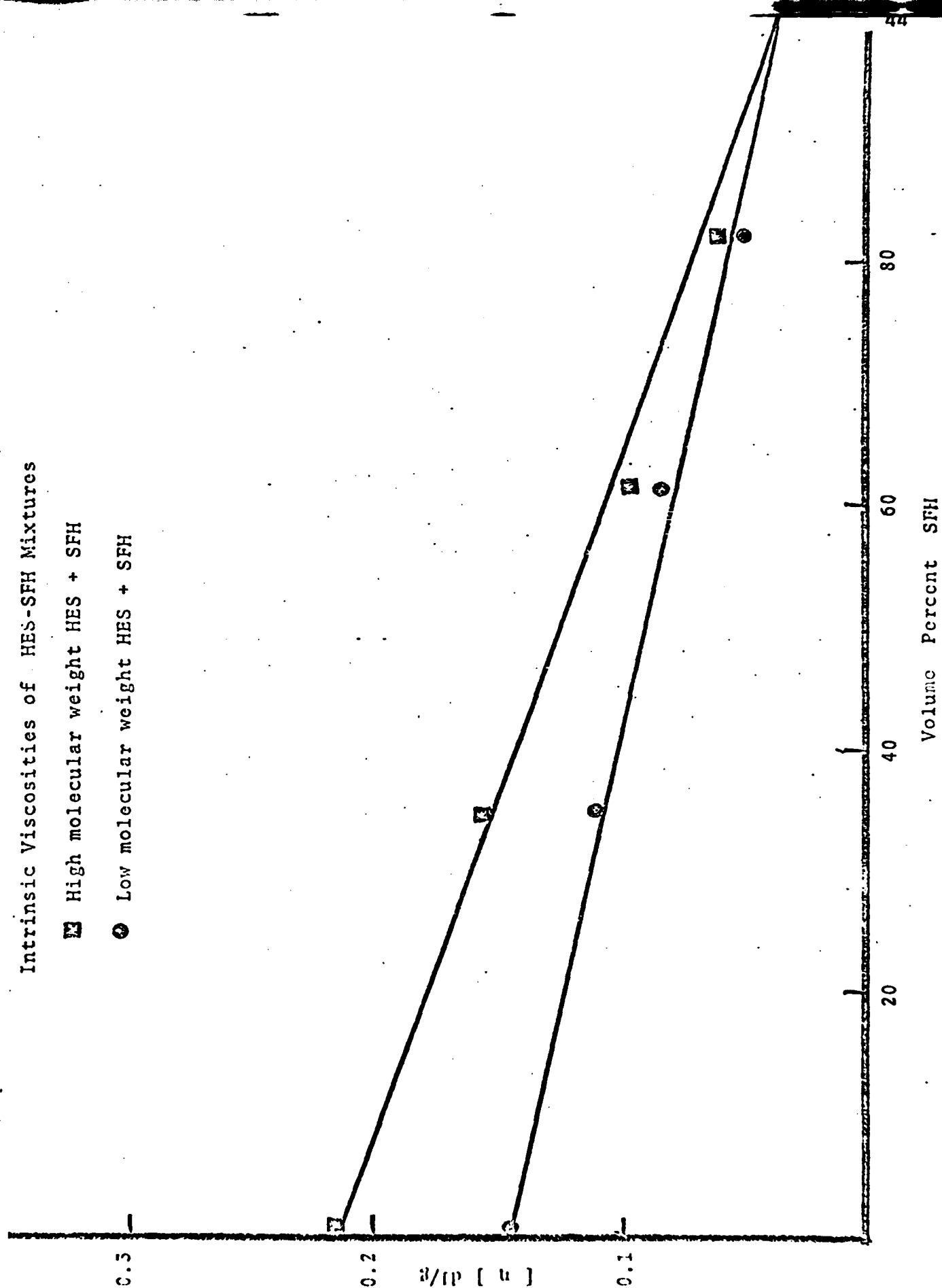
- High molecular weight HES
- Low molecular weight HES
- △ SFH

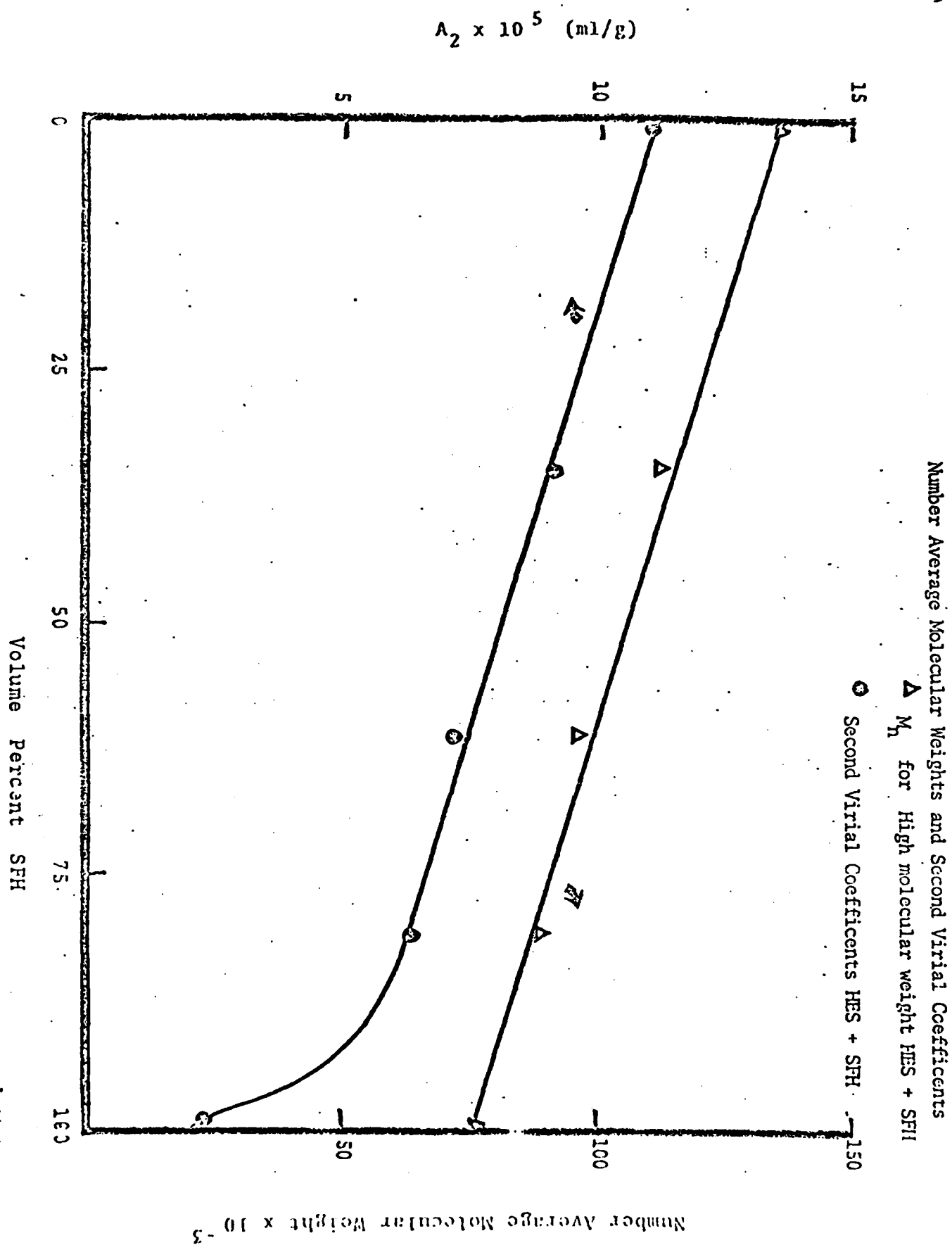


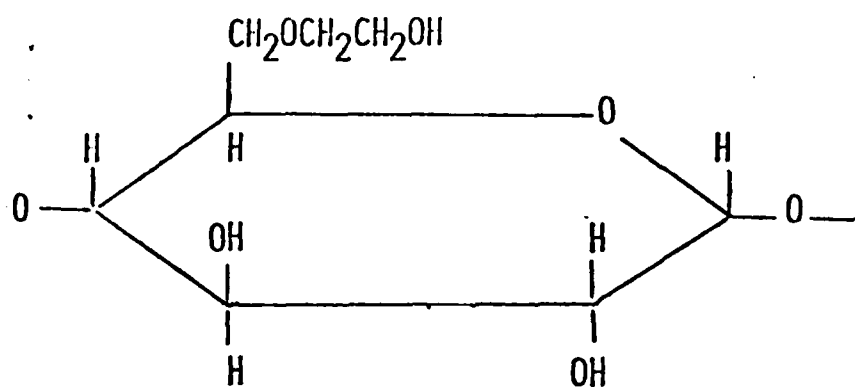
Intrinsic Viscosities of HES-SFH Mixtures

■ High molecular weight HES + SFH

● Low molecular weight HES + SFH







STRUCTURE OF A LINEAR UNIT OF HYDROXYETHYLSTARCH

H E S



CYANGEN BROMIDE



ETHYLENE DIAMINE

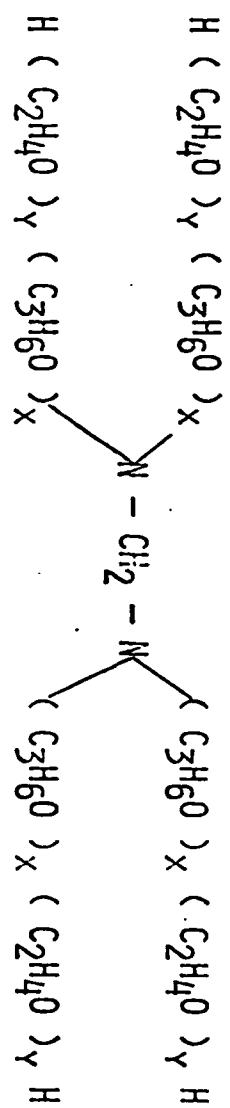


GLUTARALDEHYDE



HEMOGLOBIN

H E S - H G B POLYMER



BASIC STRUCTURE OF A TETRONIC POLYOL

