the mechanism is not a simple competition for SH or S-S groups, since the concentration of insulin added has little effect on the inhibitory concentrations of blocker. The rather interesting finding that addition of insulin prior to blocker results in greater inhibition in the fat pad has also been observed in isolated rat diaphragm muscle(1) though not in perfused rat heart(2). No obvious explanation for this is apparent. One can hypothesize that in the process of binding insulin to tissue, an increased number of binding sites are made available for the NEM or that insulin in some manner increases the permeabilty of the tissue to NEM resulting in high concentrations of NEM intracellularly.

Summary. N-ethyl maleimide, a sulfhydryl blocking agent, is capable of inhibiting the response of adipose tissue to insulin. The inhibitory mechanism is not competitive. Although the effect appears to be upon the cell surface, the binding of insulin to the tissue is not impaired.

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Relation of Molecular Size of Dextran to its Effects on the Rheological Properties of Blood.* (28198)

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In recent studies with dextran as a plasma expander in the treatment of shock(1) and in extracorporeal circulations(2), it has been reported that low molecular dextran is more effective than the higher molecular fractions in promoting and maintaining flow through the capillaries and small vessels, seemingly by lowering the viscosity of the blood and reducing the tendency of the red cells to aggregate and stagnate in the small vessels(1). The present experiments were undertaken to test the possibility that low molecular dextran actually lowers the viscosity of the blood in some way not explained by changes in the red cell concentration.

Methods and procedures. Four preparations of dextran with mean molecular weights of 15,000; 45,000; 75,000 and 250,000 (provided by Pharmachem Corp., Bethlehem, Pa.) were tested on heparinized dog blood *in vitro* for their effects on a) sedimentation rate and on b) viscosity of blood and plasma at shear rates from 0.2 to 230 sec^{-1}. Determinations of viscosity were made at 37°C with both the Brookfield cone-plate viscometer (Brookfield Engineering Laboratories, Stoughton, Mass.) (3) and the Polarad couette viscometer (on temporary loan from the Polarad Electronics Corp., Long Island City, N. Y.)(4). Although the Brookfield instrument can be set for fairly low shear rates (down to 0.3 sec^{-1}) the torque with plasma or blood at these settings is so small and the readings so near zero on the scale, that they are essentially meaningless. Hence the Brookfield viscometer

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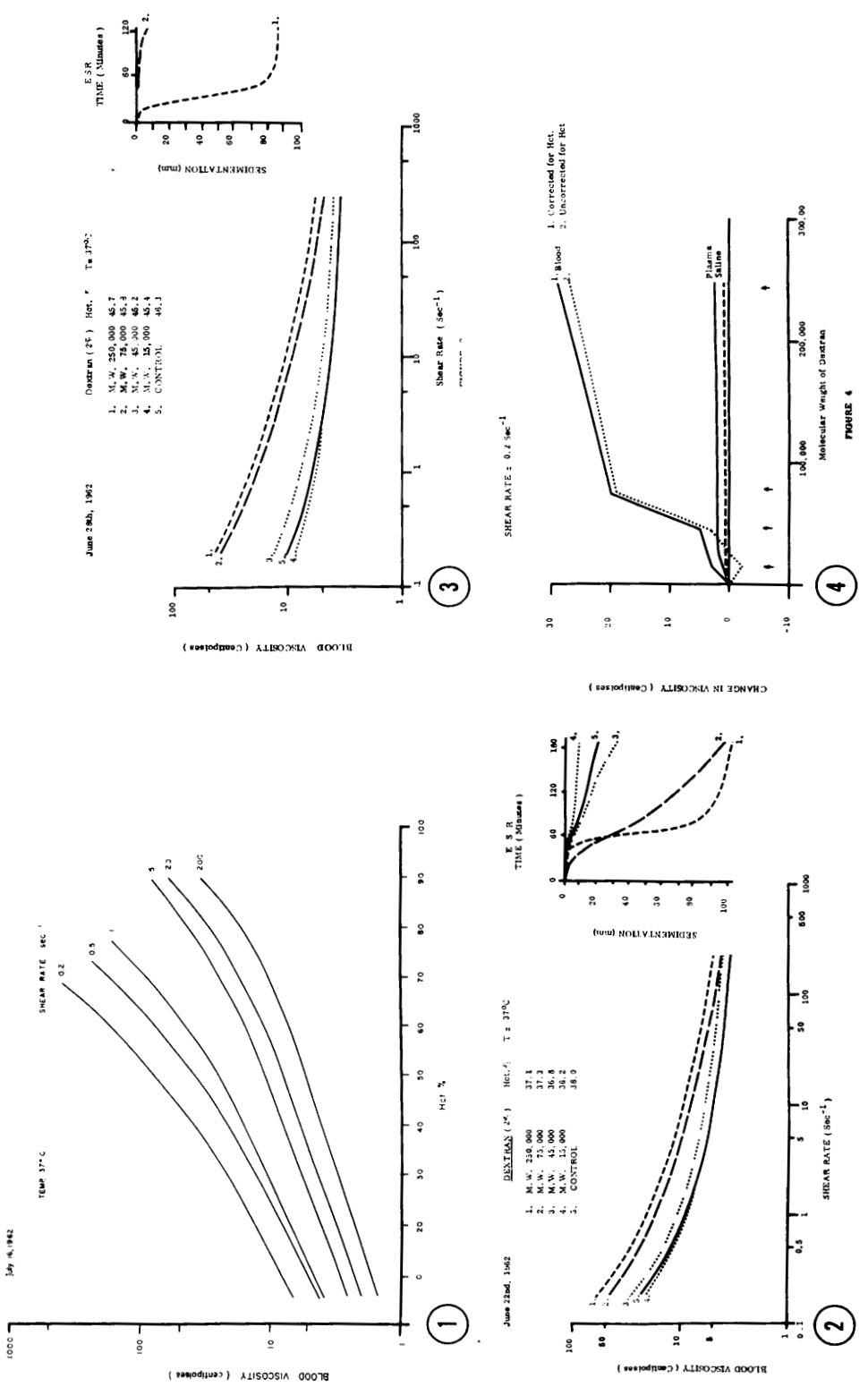
BLOOD VISCOSITY (centipoises)
SHEAR RATE sec⁻¹
TEMP 37°C
Hct %
SHEAR RATE (Sec⁻¹)
DEXTRAN (2%) Hct.% T = 37°C
1. M.W. 250,000 37.1
2. M.W. 75,000 37.3
3. M.W. 45,000 36.8
4. M.W. 15,000 36.2
5. CONTROL 38.0
E.S.R. TIME (Minutes)
SEDIMENTATION (mm)
CHANGE IN VISCOSITY (Centipoises)
SHEAR RATE : 0.2 Sec⁻¹
1. Corrected for Hct.
2. Uncorrected for Hct
Plasma
Saline
Molecular Weight of Dextran
FIGURE 4

was not used for the determinations at shear rates below 5.8 sec^{-1}. With the Polarad viscometer, reproducible results were obtained at shear rates from 2.0 to 0.2 sec^{-1}. In this range as will be seen below, the viscosity increases rapidly as shear rate is reduced(5,6).

A series of experiments was first conducted to study the effect of changes in hematocrit on blood viscosity. Heparinized dog blood samples were centrifuged at 1000 $\times$ gravity for 5 minutes and plasma was added or removed in order to prepare samples of widely different hematocrits (8 to 88%) from the same source.

Since the viscosity of blood depends to a very large extent on red cell concentration, the effects of other factors (*e.g.*, addition of dextrans of various molecular weights) cannot be readily determined unless the red cell concentration is held constant. To accomplish this, the dextrans were added to freshly drawn heparinized dog blood by replacing a suitable portion of the plasma with autologous plasma containing sufficient dextran to give a final plasma dextran concentration of approximately 2%. The hematocrit value (centrifugation in 1 ml Wintrobe tubes at 1500 $\times$ gravity for 30 minutes), sedimentation rate (ESR, using the 200-mm Westergren tubes), and the viscosity at 37°C of blood and plasma at various shear rates were determined on the control blood and on the various blood-dextran samples.

Results. The relation between red cell concentration and blood viscosity at various shear rates ranging from 200 sec^{-1} down to 0.2 sec^{-1} is shown graphically by the family of curves in Fig. 1, constructed from the results of a typical experiment. It will be apparent that the effects of changes in red cell concentration are far more pronounced at low than at high shear rates. Viscosities of the samples with hematocrits above 70% were so high at the low shear rates as to make it impossible to get readings with the Polarad viscometer. It may be seen from the results shown in Fig. 2 that 15,000 molecular weight dextran retards sedimentation rate and, at low shear rates, appears to reduce the viscosity of the blood slightly. With 45,000 m.w. dextran, both ESR and viscosity at all shear rates are somewhat increased. With 75,000 and 250,000 m.w. dextran these changes are magnified, apparently increasing with the molecular size of the dextran used. This was also borne out by separate tests made with a 450,000 m.w. dextran (Pharmacia Co., Uppsala, Sweden) which raised the viscosity so much that it was difficult to obtain readings at the lowest shear rate (0.2 sec^{-1}).

As shown in Fig. 3, similar effects were obtained in experiments with washed red cells suspended in Ringer-Locke solution. In these experiments dextran was dissolved in Ringer-Locke solution and added to the washed cell suspension by the replacement procedure described above to hold red cell percentage constant and to obtain a final dextran concentration of 2%. Again the viscosity was slightly decreased by the 15,000 m.w. dextran and increased progressively when the higher molecular weight dextrans were used. As shown in the right side of Fig. 2, the ESR at the end of 2 hours was essentially zero for the control sample as well as for the samples containing 15,000 and 45,000 m.w. dextrans; whereas the 2 hour ESR was 6 mm with 75,000 m.w. dextran and 86 mm with 250,000 m.w. dextran.

The relation between the molecular size of

FIG. 1. July 16, 1962. Heparinized dog blood. Showing relation of red cell concentration to viscosity at different shear rates. Values at 200, 20, 5, 1.0, 0.5 and 0.2 sec^{-1} were taken from viscosity curves obtained by combining measurements from both viscometers. See *Discussion*.

FIG. 2. June 22, 1962. Comparison of effects of various fractions of dextran on sedimentation rate (right side) and on blood viscosity curves (left side) of heparinized dog blood. Note slight differences in hematocrit value of control and dextran samples. For discussion see text.

FIG. 3. June 28, 1962. Comparison of effects of various fractions of dextran on sedimentation rate (right side) and on viscosity curves (left side) of washed red cells suspended in Ringer-Locke solution. There was no observable sedimentation in control sample (No. 5) or in those containing 15,000 or 45,000 m.w. dextran (samples 3 and 4).

FIG. 4. Diagram constructed from viscosities determined at lowest shear rate (0.2 sec^{-1}) obtainable with the Polarad instrument showing that increase in viscosity produced by various fractions of dextran is related to mean molecular weight dextran. It will be noted that increase in viscosity of plasma *per se* is slight compared with the change in whole blood viscosity. See text for corrections applied to results on blood.

the dextran and its effects on the viscosity of blood at 0.2 sec $^{-1}$, the lowest shear rate obtainable with the Polarad viscometer, is shown in the upper curve in Fig. 4. This Figure also shows that the effect on the viscosity of plasma *per se* (or of saline and Ringer-Locke solution) is very small indeed, even with addition of 250,000 m.w. dextran.

Discussion. Since the Brookfield and Polarad viscometers were used in different shear rate ranges, the continuous viscosity curves shown in Fig. 2 and 3 indicate that they were adequately calibrated for absolute values. The Brookfield was calibrated with water and oils K and D obtained from the U. S. National Bureau of Standards. The Polarad viscometer was calibrated only with water since it cannot be used on a non-conducting liquid.

The effects of molecular size of dextran (and other colloids) on the ESR have been carefully studied by Thorsen and Hint(7) and by Thorsen and Richter(8). In the present experiments we were interested only in a rough comparison of the effects of the various fractions of dextran on ESR in the same samples used for determination of viscosity.

The hematocrit values in the dextran samples were consistently a little below the values in the controls, (see values tabulated in Fig. 2 and 3) the difference being most pronounced with the 15,000 m.w. dextran. Hence the slight reduction in viscosity observed especially at low shear rates with this fraction (see Fig. 2 and 3), is not a direct effect of dextran on the viscosity but an indirect effect by lowering of the volume percent of the red cells. This becomes clear if the viscosity data are corrected for differences in hematocrit values by reference to the uppermost curve in Fig. 1. As shown in Fig. 4, the application of these corrections gives curve No. 1 which does not dip below the basic line with 15,000 m.w. dextran as does curve 2 plotted from the original uncorrected results.

The concentrations of dextran (2%) used in the present experiments should not theoretically have any measurable osmotic effect on the red cells. However, when the osmolarity of 4% solutions of the various fractions was tested with a Fiske osmometer (Dr. C. Chang, unpublished observations) it was found that the osmolar concentrations of 15,000 and 45,000 m.w. fractions were far in excess of values that could be ascribed to dextran alone. Also the samples were found to contain enough sodium to account for 40% of the osmolarity. Hence this and other impurities presumably account for the slightly lower hematocrit value consistently noted in the blood-dextran samples.

Summary. The viscosity of heparinized dog blood at 37°C was studied with the Brookfield cone-plate viscometer at shear rates from 230 sec $^{-1}$ down to 5.8 sec $^{-1}$ and with the Polarad couette viscometer from 2.0 sec $^{-1}$ down to 0.2 sec $^{-1}$. The relation of viscosity at various shear rates to red cell concentration was determined on samples of blood in which the hematocrit values were adjusted from 8.0% to 88%.

Several fractions of dextran with mean molecular weights ranging from 15,000 to 250,000 were tested for their effects on sedimentation rate and on the viscosity of whole blood and of washed red cells suspended in Ringer-Locker solution. Some dextran fractions contained osmotically active impurities that caused a slight lowering of the hematocrit value. When the viscosity data were corrected for differences in hematocrit values the results showed that all dextran fractions raised blood viscosity, the increase being related to the mean molecular weight of the dextran fraction added. This effect cannot be explained by the change in viscosity of the plasma.

Low molecular dextran does not reduce the viscosity of blood samples below the control value.

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Photodynamic Inactivation of Arbor Viruses by Neutral Red and Visible Light. (28199)

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The interaction of photosensitized neutral red dye and virus:cell systems was pointed out by several groups of workers(1,2,3) as a result of observations of reduced efficiency of plaque formation when infected monolayers were exposed to visible light in the presence of dye.

During control studies on the effect of this variable on Japanese encephalitis virus:cell systems, it was noted that this virus was markedly sensitive to photodynamic inactivation by neutral red and visible light even when exposed in the extracellular state. This virus thus differed from poliovirus in this respect, since it had been reported recently(4) that photodynamic inactivation occurred only when poliovirus was grown in the presence of neutral red and not when dye and mature virus particles were mixed extracellularly.

It is the purpose of this report to document these findings, to demonstrate that they apply equally to both group "A" and group "B" arbor viruses, and to point out their technical importance, as well as their possible application to production of killed virus vaccines.

Materials and methods. Preparation of stock viruses. The Nakayama strain of Japanese encephalitis virus was employed at approximately the 90th mouse passage. The strain was "purified" by 3 successive plaque passages on chick embryo fibroblasts.

Stocks were prepared from the brains of moribund suckling mice by homogenization at 10% (W/V) concentration in 10% antibody-free rabbit or calf serum saline. Some stocks were prepared by inoculation of stable monkey kidney cells (MK-2 cells kindly supplied by Dr. Robert Hull, Eli Lilly Laboratories, Indianapolis, Ind.) maintained in M199 medium and 1% calf serum. Virus stocks were clarified by centrifugation at 10,000 rpm in the high speed head of the refrigerated International Centrifuge (Model PR-1) for 30 minutes.

The following arbor viruses were also used in this study:

a. Japanese encephalitis virus, M7-1017 selected strain isolated from mosquitoes in 1957 at the Medical General Laboratory (406) and subsequently selected for heat resistance (K. Fujinaga and R. K. Gershon, unpublished experiments).

b. Yellow fever virus, 17D strain, in 2nd Medical General Laboratory (406) suckling mouse brain passage (SMB-2).

c. West Nile encephalitis virus, Egypt 101 strain, passed once in adult mouse brain, then once in suckling mouse brain.

d. St. Louis encephalitis virus, Hubbard strain, SMB-8.

e. Murray Valley encephalitis virus, strain 1, passed at Medical General Laboratory (406) once in adult mouse brain, then once in suckling mouse brain.

f. Dengue virus, type I (Hawaiian strain), passed at Medical General Laboratory (406) 11 times in adult mouse brain, then 4 times in suckling mouse brain.

g. Western equine encephalomyelitis virus, North Dakota strain, passed 6 times in adult mouse brain, then once in suckling mouse brain.

h. Eastern equine encephalomyelitis virus in second Medical General Laboratory (406) suckling mouse brain passage.

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