This procedure was repeated many times (4–8). In 21 experiments the last myocardial depression became extreme during 2 to 4 min., and revival was accomplished only by a direct, active myocardial massage and, in 11 dogs, also by injection of 25–50 mcg. of isoproterenol into an auricle rather than a ventricle.

After the last myocardial depression, with or without cardiac arrest, both artificial ventilation and carotideal cerebral circulation were given for 3 hr. During the second hour in 16 dogs saline solution was perfused into the circle of Willis at the rate of 0.5 ml./min. for the first 30 min., and in 28 dogs ATP (5.10^{-5} *M* in saline solution) was perfused into the circle of Willis at the rate of 0.5 ml./min. for the first 30 min.

RESULTS AND DISCUSSION

The repeated suppression of artificial ventilation, with carotid arteries closure, induced a high depression or silence in the EEG, with only partial spontaneous reversion after recovery of respiratory and circulatory conditions (Table I) during the time of observation. Parallel behavior was shown by the subcortical centers. The subsequent treatment with ATP, selectively perfused into the circle of Willis, improved the partial spontaneous reversion of the EEG depression, as summarized in Table I and exemplified in Fig. 1, which shows one of the best results obtained.

It is possible to note also (*a*) the lesser natural recovery in EEG pattern induced by the cardiac arrest; (*b*) the inverse correlation between the time of cerebral silence and the recovery of the EEG pattern, with or without ATP treatment; and (*c*) the lack of EEG modification by intracardiacal injection of isoproterenol during the direct active myocardial massage after cardiac arrest.

During the cerebral asphyxia, with related depression of electrical activity of the cortex, the blood samples from the jugular vein showed no modification of hematocrit and clotting-time, while the pH decreased and lactic acid increased. A rise of LDH was detectable in the cerebrospinal fluid. The recovery of respiratory and circulatory conditions corrected partially the modified values, with a possible return to normal conditions after treatment by ATP.

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Light-Scattering Investigation of Protamine Sulfate

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Abstract □ A study of the weight average molecular weight of protamine sulfate using light-scattering photometry at 436 mμ was undertaken. Results obtained indicate a molecular weight of 3600 for the free base. Dissymmetry and second virial coefficient data are discussed relative to solute-solute interactions.

Keyphrases □ Protamine sulfate—molecular weight determination □ Light-scattering spectrophotometry—analysis □ Refractive index increment—protamine sulfate solution

Protamine sulfate, a heparin antagonist, is obtained from the sperm or mature testes of fish (1) belonging to the genus *Oncorhynchus* Suckley, Salmo Linne, or Trutta Jordan et Evermann (Fam. *Salmonidae*). Since it is derived from animal sources, previous molecular weight determinations have led to the reporting of conflicting results. From end-group determinations, Phillips (2) assigned a molecular weight of 3800. Unpublished data by Callanan (3) attributed a maximal average molecular weight of 5000 to salmine on the basis of particle weight distribution.

Since the literature is void of experimental details, the discrepancy between number and weight average molecular weights caused further study of the light-scattering patterns of protamine sulfate solutions.

EXPERIMENTAL

Material—Samples of protamine sulfate powder were used (lots ONPO2E and ONPO2H, obtained from Eli Lilly Laboratories). Sulfate and nitrogen elemental analysis (4), on an anhydrous basis, gave a 17.7 and 23.3% content, respectively. Triple-distilled sterile water was used as solvent for all solutions with an apparent optical turbidity of 10^{-5} cm.$^{-1}$. All solution concentrations were calculated on the dried basis.

Light-Scattering—Refractive index increments (dn/dc) and weight average molecular weight (Mw), defined as $(n_{soln.} - n_{soln.})/c$ and the ratio $\Sigma NiMi^2/\Sigma Ni\Sigma Mi$, respectively, were obtained with a differential refractometer (Brice Phoenix) and modified dual photomultiplier type photometer (models 2000), using incident unpolarized light of 4358 Å. All samples measured gave a dn/dc of 0.180 ± 0.001 ml./g. suggesting minimum sample heterogeneity.

Temperature control was achieved by circulating thermostated water at 25 ± 0.1° through a cored light scattering cell table and jacket.

Scattering intensity was measured at 90° to the incident beam (I_{90}) relative to transmitted light (I_0) at 0°. Apparent turbidity t, and dissymmetry z, defined as the angular ratio i_{45}/i_{135} were carried out with a 40 × 40-mm. semioctagonal cell.

Light absorption at 436 mμ, depolarization, and fluorescence measurements were negligible with transmittance being greater than the limiting value of 63%.

The effect of pH on scattering was examined for solutions containing 12 × 10^{-3} g./ml. protamine sulfate in phosphate buffer, $\Gamma/2 = 0.1$, pH = 6.9–9.5. Data for all solutions were identical suggesting the absence of pH effects in this range.

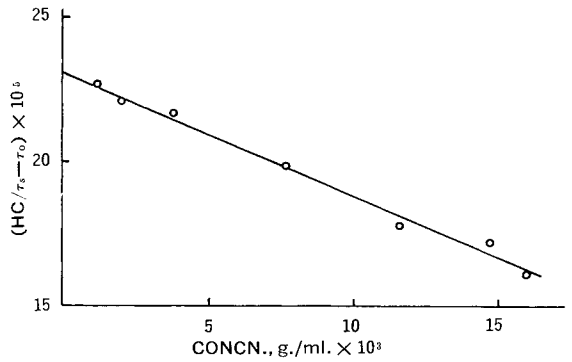

Figure 1—*Plot of* $Hc/t_s - t_0$ versus *concentration of protamine sulfate solutions at 25°.*

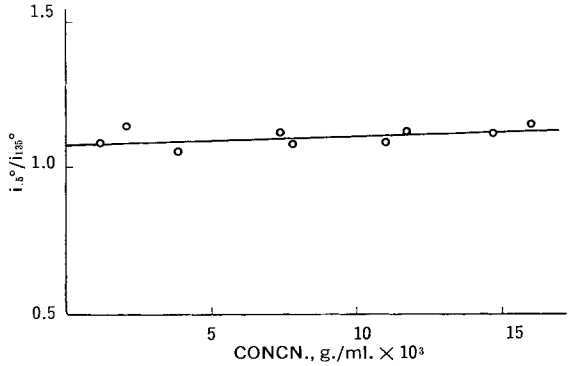

Figure 2—*Dissymmetry ratio of protamine sulfate in* H_2O *as a function of concentration at 4358Å.*

Weight average molecular weight was obtained from the expression $Hc/(t_s - t_0) = 1/M_w + 2\,Bc$, where H is an optical constant in Debye's equation (5) when turbidity is used, B is the second virial coefficient and a measure of the interactions in solution, and $(t_s - t_0)$ represents difference between apparent solution and solvent turbidity resulting in solute or excess turbidity. Using the least-squares method, extrapolation of the equation to zero concentration yields $1/M_w$.

RESULTS AND DISCUSSION

The data illustrated in Fig. 1 give a weight average molecular weight of 4350. Taking into account a 17.7% sulfate contribution, a value of 3600 is obtained for the free base. These results are in agreement with values obtained from end-group and diffusion measurements.

This correlation between number average M_N, and weight average M_w reflects a narrower distribution of molecular weights and a lesser degree of polydispersity associated with the protamine used as contrasted to the previous cited maximal value. In the case of monodisperse systems $M_w = N_N$.

Since no increase in turbidity at the lower concentrations is apparent, which in the presence of large colloidal impurities would be followed by a downward curvature, their presence is not indicated.

The negative interaction constant ($B = 2.13 \times 10^{-3}$ mole-ml./g.^{2}) suggests solute-solute interactions with water acting as a poor solvent for the system (6). This was also reflected by the low solubility (<3%) obtained at 25°. Negative second virial coefficients, in salt free solutions, have also been reported for other proteins suggesting long range intermolecular attractive forces are operative (7).

The progressive increase of $Hc/(t_s - t_0)$ with decreasing concentration is in accord with the molecular-binding interpretation (8) that this ratio increases when net forces acting between two components, *i.e.*, solute-solvent, is repulsive and results in negative binding.

Forward i_{45} to backward i_{135} scattering was examined by measuring the angular distribution at angles symmetrical to 90° as a function of increasing concentration. As can be seen in Fig. 2, dissymmetry data show a degree of concentration dependency suggesting solute-solute interactions are preferred. The extrapolated value $(Z)_{c=0} = 1.08$ indicates absence of deficit scattering at 90° denoting that the function $Hc/(t_s - t_0)$ against concentration represents a valid two-component extrapolation for the system with approximately equal forward to backward scatter.

The dissymmetry noted is further evidence that protamine is a relatively small particle since the presence of a large entity would increase scattering and Z ratios. Its low order of magnitude does not permit particle shape determination since at dissymmetry values lower than 1.1 graphical interpretation methods of Z against characteristic dimension coincide for coils, rods, and spheres (9). This suggests that protamine is much lower than 436 Å. in dimension.

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