

Approximately twice the D-glucaric acid was removed when the volume of urine was doubled. These two findings confirm that the inhibitor of β -glucuronidase is D-glucaro-(1 \rightarrow 4)-lactone.

Diazepam, chlordiazepoxide, carbamazepine, ethosuximide, methsuximide, phenytoin (and 5-(*p*-hydroxyphenyl)-5-phenylhydantoin, its main metabolite), phenobarbitone and primidone did not interfere with the assay when added to the urine. Similarly, excess glycine, (+)-tartaric acid, L-ascorbic acid, D-glucuronic acid and increasing the concentration of urea in the urine did not interfere with the assay.

The results presented support the use of the enzymatic assay of urinary D-glucaric acid as an index of hepatic enzyme induction in patients receiving antiepileptic drugs.

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The micellar weights of the series dioctyl-to-didodecyl phosphatidylcholine

The effect of prolonged ultrasonication on aqueous sols of natural phosphatidylcholine (PC) has been reported independently by Attwood & Saunders (1965), Saunders (1966) and Huang (1969), and while there is agreement on a micellar weight of about 2.0×10^6 , differing opinions on the structure of the micelle are held. Huang argues for a bileaflet structure enclosing a core of solvent, whereas Saunders proposes that the particles are fragments of bilayers, the sheet of molecules being held firmly folded by head group interactions between the phosphate and choline ions.

The micellar weights of a series of synthetic dioctyl-to didodecyl-phosphatidylcholines (C_8 to C_{12} PCs) supplied by Dr. F. C. Reman of the Biochemistry Department University of Utrecht have been determined by light scattering. Reman, Demel & others (1969) have reported on the haemolytic activity of this series.

Two light scattering instruments were used. One used a low power He-Ne laser ($\lambda = 632.8$ nm) as the light source (Pugh, 1970; Pugh & Saunders, 1971) and the other had a mercury lamp ($\lambda = 546.1$ nm).

The samples were dissolved in a small amount of moist ether, shaken in water and the ether removed by treatment in a vacuum rotary evaporator at room temperature (20°), followed by bubbling with nitrogen. All samples were sonicated for 90 min at the maximum cavitation frequency (about 20 kHz) by a 60W Mullard generator with a titanium probe. The samples were immersed in an ice bath and nitrogen

was gently blown over the surface during the sonication. Large particles and fragments of titanium were removed by centrifugation at 27 000 *g* for 1 h and the sols were filtered into the light scattering cell through Millipore membranes with a pore diameter of 0.45 μm . Water was obtained from an Elgastat deionizer containing macroreticular resins and fitted with sterilizing membrane filters. The conductivity was less than $1 \times 10^{-5} \Omega^{-1}\text{m}^{-1}$. The Millipore membranes were boiled before use in several changes of water to remove the surfactant material added during manufacture. The refractive index of the filtered sols was measured by a Hilger-Watt differential refractometer.

The scattered light intensity was measured over a range of angles from 30° to 150° relative to that of a standard Perspex block and S_θ calculated as

$$S_\theta = \frac{\text{Scatter of sol at } \theta}{\text{Scatter of block at } 90^\circ} \cdot \frac{\sin \theta}{1 + \cos^2 \theta \cot^2 \phi}$$

ϕ = angle between plane of polarization of laser beam and horizontal. Corrections were made for the solvent scatter and the reflections of light from the exit face of the cell.

It has been suggested (Pugh & Saunders, 1971) that the usual equation relating S_θ to the weight average molecular weight M_w ,

$$\frac{1}{M_w} = K' \cdot \left(\frac{c}{S_\theta} \right) \quad c, \theta \rightarrow \text{zero}$$

may, with advantage, be replaced by

$$\frac{1}{M_w} = K \cdot \left(\frac{\Delta n}{S_\theta} \right) \quad \Delta n, \theta \rightarrow \text{zero}$$

where n_0 = refractive index of solvent; dn/dc = refractive index increment; Δn = refractive index difference between solution and solvent; N_A = Avogadro's number; G = constant for standard Perspex block

$$K = \frac{2\pi^2 n_0^2 (dn/dc)}{\lambda^4 N_A G}$$

Normally a plot of $(K\Delta n/S_\theta)\Delta n \rightarrow \text{zero}$ against $\sin^2(\theta/2)$ is a straight line; deviation from linearity is due either to polydispersity or to the dimensions of the particles being outside certain limits. The plots for all the compounds except $C_8\text{PC}$ became linear only at high angles which is characteristic of a type of polydispersity

Table 1. M_w Values for PCs ultrasonically dispersed in H_2O .

PC	$M_w \times 10^{-6}$	Aggregation No.	Notes
Egg	2.04		1
C_8^*	0.279	533	2
C_9	0.278	531	3
C_9	1.96	3560	
C_{10}^*	4.00	6910	
C_{10}	3.90	6740	
C_{11}	3.41	5630	
C_{12}	2.044	3220	

* Measurement at 546.1 nm, others at 632.8 nm.

1. Attwood & Saunders (1965), Huang (1969) 2.0×10^6 .

2. Determined by extrapolation to zero angle.

3. Determined by S_{90} measurement with Cabannes and disymmetry corrections.

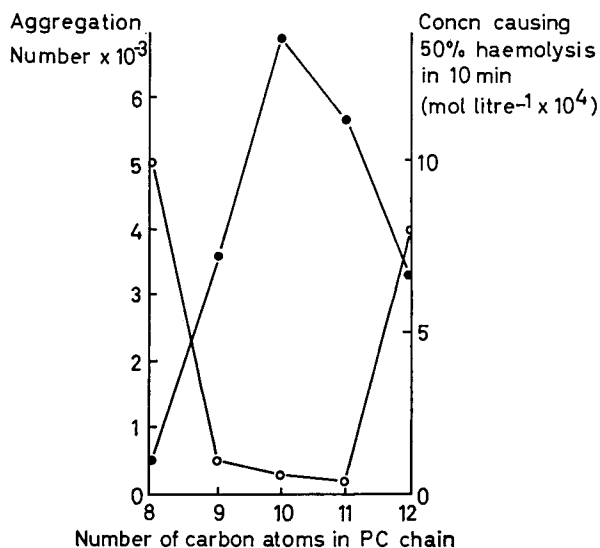


FIG. 1. Effect of chain length on aggregation number and haemolytic properties of PCs. ● Aggregation number ($\times 10^3$). ○ Concentration causing 50% haemolysis in 10 min ($\text{mol litre}^{-1} \times 10^4$).

noted by Kratochvil (1965) in which a mainly monodisperse system is contaminated by relatively few much larger particles. Since the concentration of the coarser particles is negligibly small, the asymptote at high $\sin^2(\theta/2)$ values may be taken as being due to the monodisperse, smaller particle system only.

The M_w values for the smaller particles are given in Table 1. They show an interesting correlation with the haemolytic activity as determined by Reman (Fig. 1). He makes the point that the distribution of the phospholipid between the aqueous phase and the cell surface is an important factor in haemolysis. It is seen from the figure that the greatest haemolytic activity corresponds with the largest micellar sizes, indicating that the governing factor for this activity in this series of compounds is the strength of the non-polar interaction between the phospholipid and the membrane constituents, the larger micelles providing a more extensive non-polar core in which membrane constituents may be solubilized.

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