then increase again, provided that the solute is homogeneous with respect to $(dn/dc_2)_1$ and α_2; if the solute is heterogeneous in either respect, a non-zero minimum of the excess scattering will be reached.

We have used this method to measure the selective interaction (in this case, exclusion) of hyaluronic acid (component 3) with bovine serum albumin (component 2) and have obtained results that agree with partition measurements[6] and with osmotic measurements[2,7].

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Phosphorylase a/b Ratio in the Lamprey Heart

Cyclostome branchial hearts are known to contain large amounts of endogenous catecholamines[1] and to be markedly insensitive to added adrenaline[2,3]. There is considerable evidence to suggest that the process whereby the sympathomimetic amines normally enhance the force of cardiac contraction involves the transformation of the enzyme phosphorylase from the *b* to the *a* form[4,5]. The lack of sensitivity of cyclostome hearts to added adrenaline or noradrenaline might therefore be due to the endogenous amines having already activated the conversion of the phosphorylase enzyme from the *b* to the *a* form so that even in the absence of any added adrenaline or noradrenaline the enzyme phosphorylase would exist mainly in the *a* form. This possibility was investigated using hearts isolated from freshly decapitated lampreys (*Mordacia*). After five minutes immersion in aerated (95 per cent O_2 + 5 per cent CO_2) modified Ringer solution[6] at 22° C, under which conditions isolated hearts will beat spontaneously for many hours, the hearts were rapidly frozen and the phosphorylase activity determined as described previously[7], using tissue dilutions of 1/150 (w/v) and rabbit liver glycogen (Nutritional Biochem. Lab.) which had been purified as described by Krebs *et al.*[8]. The reaction mixture was incubated at 25° C.

Typical results are displayed in Table 1 where it is shown that approximately half the phosphorylase enzyme present in lamprey hearts is in the *a* form. Comparison of these results with those obtained from other similar experiments in which freshly isolated toad (*Bufo marinus*) hearts were used indicates that the percentage of the enzyme phosphorylase in the *a* form is approximately the same in toad and lamprey hearts. These results are summarized in Table 1. Lamprey hearts are known to contain approximately 50 μg catecholamines per g[1]; toad hearts contain approximately 1·25–1·6 μg/g[9]. It therefore seems unlikely that the endogenous catecholamines of lamprey hearts participate to any marked extent in the natural regulation of the percentage of the enzyme phosphorylase which is present in the *a* form.

Perfusion of isolated toad hearts with Ringer solution containing 4 μg/ml. adrenaline ('Hermette', D. Bull and Co.) or noradrenaline ('Levophed', Winthrop Laboratories) produces a marked positive inotropic and chronotropic response. The data summarized in Table 1 indicate that these inotropic and chronotropic changes are accompanied by the conversion of almost all the phosphorylase enzyme to the *a* form. These same concentrations of adrenaline and noradrenaline failed to produce either a chronotropic or inotropic response from isolated lamprey hearts. Table 1 shows that 4 μg/ml. of either adrenaline or noradrenaline did not produce any marked change in the percentage of the phosphorylase enzyme which was present in the *a* form in lamprey hearts. 100 μg/ml. adrenaline and 400 μg/ml. noradrenaline produced small positive chronotropic and inotropic responses from isolated lamprey hearts. Table 1 shows that these same concentrations of adrenaline and noradrenaline increased the percentage of the phosphorylase enzyme present in the *a* form.

Table 1. Percentage of Phosphorylase Enzyme Present in the 'a' Form in Isolated Lamprey and Toad Hearts

Experiment	Lamprey		Toad	
	% *a*	Inotropic action	% *a*	Inotropic action
Control	63		58	
	54		62	
	57		55	
	35		63	
Adrenaline 4×10^{-6}	69	−	88	++++
	62	−	96	++++
Adrenaline 1×10^{-4}	83	++	98	++++
	69	+	98	++++
Noradrenaline 4×10^{-6}	46	−	85	++++
	48	−	92	++++
Noradrenaline 4×10^{-4}	61	+		++++
	62	+	98	++++
Isoprenaline 4×10^{-6}	89	+++	100	++++
	95	+++	95	+++
Isoprenaline 5×10^{-5}	92	+++		
	92	+++		

Where '−' denotes the absence of inotropic activity; '+' denotes an arbitrary unit of positive inotropic activity. Results of individual experiments are shown.

During the course of these experiments it was observed repeatedly that 4–50 μg/ml. isoprenaline ('Isuprel', Winthrop Laboratories) evoked a marked positive inotropic and chronotropic response from isolated lamprey hearts. In Table 1 it is shown that isoprenaline was more effective than either adrenaline or noradrenaline in converting the enzyme phosphorylase in lamprey hearts from the *b* to the *a* form.

In conclusion these results suggest that a parallelism exists between the ability of certain amines to exert a positive inotropic effect on isolated lamprey hearts and their ability to activate the conversion of the enzyme phosphorylase from the *b* to the *a* form. It seems unlikely that the endogenous catecholamines present in lamprey hearts participate in the natural regulation of the phosphorylase *a*/*b* ratio in this tissue, or that the insensitivity of these hearts to added adrenaline and noradrenaline can be accounted for simply in terms of the percentage of the enzyme which naturally occurs in the *a* form.

The expenses of this investigation were defrayed by a grant-in-aid from the Life Insurance Medical Research Fund of Australia and New Zealand.

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