separate receptor sites for NA and f-THF. However, the fact that there is considerable mutual potentiation of these two substances[5] suggests interaction between the two sites of action.

Although these results clearly indicate an antagonism between anti-epileptic drugs and f-THF and NA on the respiratory rate of a brain preparation, the relevance of this to the *in vivo* situation is not known.

Department of Pharmacology,
Guy's Hospital Medical School,
London, England

R. G. SPECTOR

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Effects of potential gradient on the electrophoretic mobility of human blood platelets in the presence of ADP or noradrenaline

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IN THROMBOGENESIS platelets stick to the wall of a blood vessel where they attract other platelets, and because they normally carry a net negative charge and are mutually repellent it is thought that in thrombogenesis or platelet aggregation *in vitro* the platelet surface charge is somehow reduced. Such a change should be reflected in the mobility of the platelets in an electric field, and electrophoresis has been used to measure it.

The effects of various aggregating agents on the electrophoretic mobility of platelets from healthy people and patients with various diseases have been studied by several groups of workers, but the results are conflicting. According to Hampton and Mitchell[1] low concentrations of adenosine diphosphate (ADP) or noradrenaline increase the mobility of normal platelets while higher concentrations reduce it. These workers[2,3] claimed that the concentrations of ADP and noradrenaline required to produce the maximum increase in mobility are decreased in various acute illnesses, whereas in arterial diseases the sensitivity to ADP is increased but that to noradrenaline is normal. Gröttom,[4] however, was unable to confirm the claim that low concentrations of either aggregating agent increase mobility: he maintained that the two compounds can only decrease mobility. Furthermore he was unable to demonstrate any differences between healthy people and patients with vascular disease in the way their platelet mobilities responded to the two aggregating agents. Rutty and Vine,[5] attempting to resolve these contradictory claims, obtained results on normal platelets which they said supported those of Hampton and Mitchell.[1] All three sets of results on normal platelets are given in Table 1.

Electrophoretic studies on platelets offer much promise in the diagnosis of disease and in the elucidation of mechanisms by which platelet behaviour is changed, but the present situation of contradictory claims is disappointing. As Turpie, McNicol and Douglas[6] have opined in their review of this field "further work is required to resolve this issue".

Hampton and Mitchell[1–3] used platinum electrodes and a capillary tube micro-electrophoresis apparatus developed by Bangham *et al.*[7] and manufactured by Rank Bros., Cambridge, England, whereas Gröttom[4] used copper–copper sulphate or silver–silver chloride electrodes and a flat rectangular cell apparatus developed by Ruhenstroth-Bauer[8] and made by the Zeiss Co., Oberkochen, Germany. Rutty and Vine[5] used the Rank model and silver–silver chloride electrodes. All three groups used citrated platelet-rich plasma (PRP) diluted with 9 vol. of citrated platelet-poor plasma (PPP). Although differences in apparatus and methodology have been emphasized and discussed by several workers[2,3,6] no explanation has emerged to reconcile these conflicting claims.

Having only a capillary tube microelectrophoresis apparatus I was unable to repeat Gröttom's

TABLE 1. THE REPORTED EFFECTS OF ADP AND NORADRENALINE ON THE ELECTROPHORETIC MOBILITY* OF HUMAN PLATELETS

Reference	μg ADP/ml (final conc.)			
	0·005	0·05	0·5	5
Hampton and Mitchell[1]	107	114	94	
Gröttom[4]	98·2	98·2	87·2	69·8
Rutty and Vine[5]	98·0	103·5	93·1	
	μg Noradrenaline/ml (final conc.)			
	0·005	0·05	0·5	5
Hampton and Mitchell[1]	102	108	92	
Gröttom[4]		99·0	95·4	86·3

* Mobilities are expressed as percentages of their control values.

experiments,[4] but I thought it worthwhile to determine whether differences in technique could produce in a single apparatus results comparable with those of both Gröttom[4] and Hampton and Mitchell.[1]

Nine vol. of blood were withdrawn from the antecubital veins of overtly healthy human volunteers of either sex into sterile disposable syringes containing 1 vol. of 3·8% sodium citrate solution. PRP was prepared by centrifugation in a Mistral 6L machine at 1600 rev/min for 4 min at 37°, and PPP was prepared from some of the PRP by centrifugation at 3000 rev/min for 15 min, again at 37°. One vol. of PRP was immediately added to 9 vol. of PPP, and the mixture was kept at room temperature for 1–2 hr before the start of each experiment. The apparatus used was a capillary tube microelectrophoresis model (supplied by Rank Bros) thermostatically controlled at 25°. Adenosine 5′-diphosphate disodium salt (Sigma Chemical Co.) and noradrenaline bitartrate (Koch–Light Laboratories) were dissolved in 0·9% saline (prepared from triple-distilled water) and 1 vol. was added to 100 vol. of the PRP–PPP mixture. The final mixture was introduced into the electrophoresis cell and 5 min later the mobility of the platelets was determined at the stationary level. The transit times across one graticule division (28·7 μ) were determined for at least 10 platelets, and the mean value was used to compute the mobility, expressed in μ/sec/V/cm. To avoid polarization the electrode polarities were reversed after each determination.

In a series of experiments on single plasma samples from 12 different individuals the effects of 4 conc. (final values 0·005, 0·05, 0·5 and 5 μg/ml) of ADP on platelet mobility were measured at potential gradients of 2, 3, 4 or 6 V/cm, 3 individuals being used for each potential gradient.

Conductivity and viscosity values were not determined in these experiments as earlier experiments had shown that the addition of 1 vol. of saline (or saline containing ADP or noradrenaline in the appropriate concentrations) to 100 vol. of the PPP–PRP mixture had no effect on these values.

The mobilities, expressed as percentages of their respective controls, are given in Table 2. It is clear from these results that at low concentrations of ADP the potential gradient used had a marked effect on the calculated mobility. At 2 and 3 V/cm the results agree with those of Hampton and Mitchell[1] whereas at 4 and 6 V/cm they agree with those of Gröttom.[4]

Similar experiments on plasmas from 2 groups of 3 volunteers were made with noradrenaline at potential gradients of 3 and 4 V/cm, and again marked differences were obtained at the lower concentrations (Table 2). Again the results at 3 V/cm agree with those of Hampton and Mitchell[1] whilst those at 4 V/cm compare with those of Gröttom.[4]

Hampton and Mitchell[1–3] reported using a gradient of 2·66 V/cm and Gröttom[4] a current of 5 mA. Assuming that the cross-section of the electrophoresis chamber in the Zeiss instrument is 0·1 cm² and that the specific resistance of normal plasma is 84 Ω per cm cube then it follows from Ohm's Law that the potential gradient used by Gröttom was 4·2 V/cm. Rutty and Vine used gradients of 3·1–3·3 V/cm.*

Finally it was decided, albeit naïvely, to confirm that the electrophoretic mobility of platelets in the absence of aggregating agents is independent of potential gradient. Six volunteers were used in these experiments, in which mobilities were determined at 2, 3, 4 and 6 V/cm and expressed as percentages of the value obtained at 2 V/cm. The mean results (±S.E.) at 3, 4 and 6 V/cm were 98·3 ± 1·9, 99·8 ± 0·7 and 100·3 ± 1·7 respectively.

* Rutty, private communication.

TABLE 2. THE EFFECTS OF ADP AND NORADRENALINE AND VARIOUS POTENTIAL GRADIENTS ON THE ELECTROPHORETIC MOBILITY* OF HUMAN PLATELETS

		μg ADP or Noradrenaline/ml (final conc.)		
	0·005	0·05	0·5	5
ADP	2 V/cm			
	107·1 ± 1·4†	109·9 ± 3·3	88·6 ± 4·2	77·3 ± 4·6
	3 V/cm			
	108·7 ± 0·8	111·5 ± 2·2	89·7 ± 1·9	76·1 ± 2·0
	4 V/cm			
	97·5 ± 1·1	96·3 ± 1·0	87·1 ± 3·9	73·6 ± 2·2
	6 V/cm			
	94·7 ± 0·5	88·2 ± 1·4	82·1 ± 2·8	75·1 ± 1·7
Noradrenaline	3 V/cm			
	106·5 ± 2·4	111·9 ± 3·6	90·0 ± 2·2	87·8 ± 0·4
	4 V/cm			
	96·5 ± 2·7	86·9 ± 5·8	80·9 ± 4·6	77·0 ± 2·1

* Mobilities are expressed as percentages of their control values.
† Mean ±S.E.

It has always been assumed that electrophoretic mobility, expressed in μ/sec/V/cm, is independent of potential gradient, but clearly this is not so for platelets in plasma containing low concentrations of ADP or noradrenaline. The results reported here indicate that it is possible to increase or decrease the mobility of platelets in plasma containing low concentrations of ADP or noradrenaline simply by altering the potential gradient.

Hampton and Mitchell[1] have suggested that in low concentrations ADP is adsorbed onto the platelet surface thus increasing electro-negativity and mobility, and that at some critical concentration of ADP on the platelet surface calcium ions also are adsorbed, thus reducing the platelet negative charge and mobility.

Presumably the readiness with which ADP is adsorbed onto the platelet surface is a function of plasma ADP concentration and of the platelet surface charge, changes in which might be induced by increasing field strength, thus facilitating the adsorption of ADP and later that of calcium ions.

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Glaxo Research Limited,
Sefton Park,
Stoke Poges,
Buckinghamshire, England

E. G. TOMICH

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