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The 'Electronic platelet aggregometer'

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We have invented a device which can be used to study platelet behaviour in whole blood as well as platelet rich plasma. An evaluation of this 'electronic aggregometer' will be the subject of a communication presented by us at this meeting. In this demonstration we will deal with the principle of its operation and method of use.

Citrated (or heparinized) blood or plasma samples (1 ml) are pipetted into siliconized glass cuvettes (of the type used in standard aggregometers) and placed into heated holders (37°C) and stirred at 600 rpm with a 'flea' magent. A perspex cap that fits on top of the cuvette holder holds two electrodes – 0.25 mm diameter platinum wires 1.5 cm long, separated by approximately 1 mm. The electrode assembly projects into the sample to a depth of 1 cm and is energised by an oscillator generating a 15 kHz sine wave with an amplitude of 100 mv, which is passed through the blood between the electrodes. Electron microscopy showed that during the initial contact with the sample, the electrode becomes coated with a platelet monolayer. In the presence of aggregating agents, however, platelets stick to the monolayer and progressively cover the electrode. Resultant changes in conductance cause a change in the excitation voltage across the cell and this is amplified, rectified and filtered before being fed via an outlet socket to any suitable chart recorder. The trace thus obtained is in many respects identical to that obtained with an optical aggregometer.

After aggregation has occurred the electrode assembly is removed and cleaned with a piece of tissue. The cuvette is rinsed with saline and the apparatus is then ready for another sample.

The operation of the machine depends upon efficient control of temperature and stirring rate, but it is extremely simple to use and is suitable for measuring the aggregation of platelets within 1–2 min of obtaining the blood sample. It is, therefore, well adapted for assay of labile endogenous hormones such as prostacyclin. The device is novel, although a similar principle has been used to measure clot formation (Amiram, 1970) in blood.

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Influence of mode of blood sampling on the immunoreactive insulin concentration in serum of *Bordetella pertussis*-treated mice

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Pertussis vaccine induces hyperinsulinaemia in mice and rats (Gulbenkian, Schobert, Nixon & Tabachnick, 1968) and augments the hyperinsulinaemia induced by various stimuli (Sumi & Ui, 1975). Hyperinsulinaemia was observed also in *B. pertussis* infection in mice (Pittman, Furman & Wardlaw, unpublished). A

somewhat puzzling finding has been the absence of marked hypoglycaemia in *pertussis*-treated mice, despite very high insulin levels (Gulbenkian *et al.*, 1968; Pittman *et al.*, unpublished). This communication reports an effect of blood sampling method on the measured level of serum insulin of *pertussis*-treated mice. Female mice (HAM/ICR strain, 3–4 weeks old, 14–25 g) were treated with either, (i) *B. pertussis* vaccine (0.5 ml of 10 International Opacity Units i.p.) or, (ii) a sub-lethal dose of *B. pertussis* viable organisms (Strain 18–323, 5×10^4 colony forming units, administered intranasally under ether anaesthesia). Control mice were inoculated with the diluents. Fourteen days later, blood was removed from freely-fed mice by either heart puncture under ether anaesthesia or by decapitation without the use of ether. Serum immunoreactive insulin (IRI) was measured by the method of Hales & Randle (1963) and serum glucose was determined enzymically.

The table shows that serum IRI concentrations in *pertussis*-treated mice were not significantly different from control concentrations when blood was collected by decapitation. However, blood sampling under ether anaesthesia appears to have provided a stimulus which induced hyperinsulinaemia in *pertussis*-treated animals. Ether anaesthesia was used by Gulbenkian *et al.* (1968) and by Pittman *et al.* (unpublished). These observations appear to be consistent with the findings of Sumi & Ui (1975) that adrenaline induces marked hyperinsulinaemia in *pertussis*-vaccinated but not in control rats. Possibly adrenaline released by ether anaesthesia may have produced the hypersulinaemia in *pertussis*-treated mice.

A significant, although not severe, hypoglycaemia was found in the *pertussis*-infected but not in *pertussis*-vaccinated mice. The lack of marked hypoglycaemia in the *pertussis*-treated animals may be explained by the hyperinsulinaemia not appearing until provoked by ether anaesthesia.

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Table 1 Effect of mode of collection of blood on the serum IRI and glucose levels in *pertussis*-treated mice

	Blood collected by			
	Decapitation		*Heart puncture, ether anaesthesia*	
Treatment	*IRI (μU/ml)*	*glucose (mg/dl)*	*IRI (μU/ml)*	*glucose (mg/dl)*
Control	70 ± 10(7)	174 ± 9(7)	37 ± 7(10)	166 ± 9 (9)
Pertussis vaccine	52 ± 10(6)	148 ± 22(6)	186 ± 22** (9)	178 ± 11 (9)
Control	41 ± 13(5)	172 ± 10(5)	33 ± 6(10)	177 ± 6(10)
Pertussis infection	69 ± 8(6)	126 ± 6*(6)	160 ± 19**(10)	141 ± 7**(10)

(Values are mean ± s.e. mean. Numbers in brackets indicate number of observations. Significance of differences (control v. *pertussis*-treated) was determined by the Mann-Whitney U test. *$P \leqslant 0.05$; **$P \leqslant 0.01$)

An evaluation of hind paw oedemas in the guinea pig

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The discovery of new anti-inflammatory drugs has relied largely on the use of hind paw oedema models using the rat as the experimental animal. Ultra violet-induced erythema in the guinea pig is an exception although its relevance has never been fully understood. Other models of inflammation in the guinea pig have been reported although hind paw oedemas equivalent to those described in the rat have not been extensively evaluated. Consequently we have undertaken an investigation of the sensitivity of the guinea pig to various inflammatory mediators and irritants injected into the hind paw. Furthermore, the effects of a variety of mediator antagonists and anti-inflammatory drugs have been examined.

Male Dunkin Hartley guinea pigs (180–200 g) were used in groups of 5 throughout these experiments unless otherwise stated. Histamine and bradykinin (1–10 μg/paw) produced dose related paw oedema maximal at 15 min when injected via the subplantar (s.p.) route. 5-Hydroxytryptamine (5-HT, 10-400 μg/paw) and compound 48/80 (10–50 μg/paw) also produced a maximal oedema at 15 min but the responses were not as great as for histamine or