by the addition of 5 volumes of ice-cold 0·2 M NaH_2PO_4 solution and the reaction tubes transferred to ice.

The [^{14}C]-acetylcholine is extracted by adding 10 volumes of 3-heptanone containing 2·5 mg/ml of Kalignost (Fonnum, 1969). All of the organic layer is removed for liquid scintillation counting. The [^{3}H]-acetate is extracted into toluene/iso-amyl alcohol after the incubation solution had been acidified with 0·2 N HCl (Potter, 1967). The scintillation fluid used was that described by Buckley & Heaton (1968). The 2-channel liquid scintillation spectrometer (Packard Tri-carb 3002) was set so that one channel counted [^{14}C] with an efficiency of 20% and [^{3}H] with an efficiency of 0·1%. The other channel counted [^{3}H] with a 12% efficiency and [^{14}C] with a 60% efficiency. [^{14}C] or [^{3}H] n-hexadecane was used as an internal standard.

Results will be presented to illustrate the use of the method for cholinesterase and choline acetylase determination in freeze-dried sections of rat nervous tissue.

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Determination of the iontophoretic release of adenosine diphosphate from micropipettes

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The application of ionized substances to blood vessels in the microcirculation by micro-iontophoresis was first described by Duling, Berne & Born (1968). This method permits accurate control of the site so that reactions of different types of small vessel can be determined. We describe here a technique for quantitating the dose by measuring the iontophoretic release from micropipettes of substances available at high specific radioactivities.

The adhesion of circulating platelets in blood vessels may be initiated by adenosine diphosphate (ADP) released from damaged cells (Born, 1962). We have, therefore, been investigating the mechanism of this adhesion by applying ADP iontophoretically to small vessels in the hamster cheek pouch (Begent & Born, 1970a, b). As a step towards determining the lowest effective concentration of ADP, the rate has been measured at which radioactive ADP is released from micropipettes by iontophoretic currents. Micropipettes (tip 1–3 μM) were filled with 10^{-2}M [G-^{3}H]-ADP (specific activity, 44·3 Ci/mol, purity >95%) and connected by an external circuit to a silver silver-chloride reference electrode.

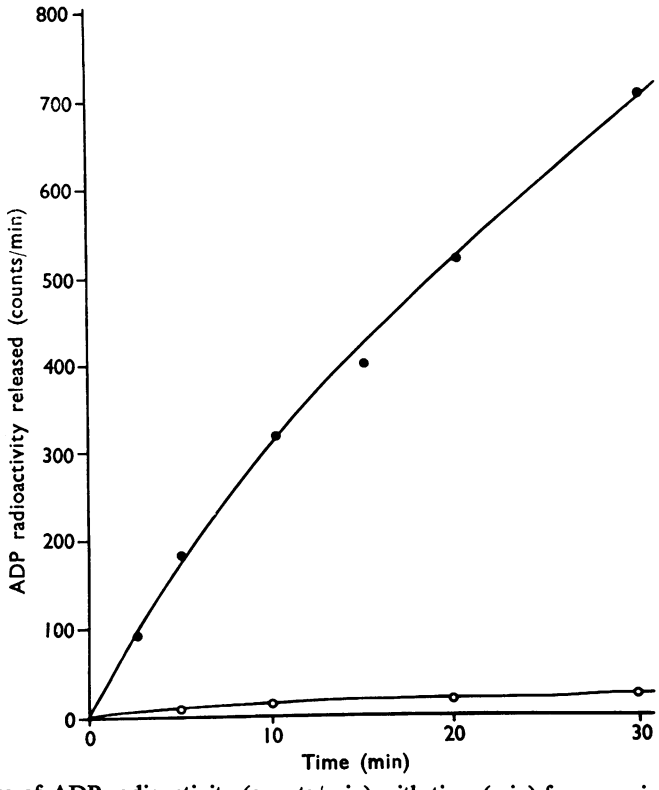

FIG. 1. Release of ADP radioactivity (counts/min) with time (min) from a micropipette (resistance 20 MΩ) filled with 10^{-2}M tritiated ADP, without (○——○) and with (●——●) a current of 300 nA.

A micropipette and the electrode were immersed in 0·5 ml saline in a counting vial. The micropipette was inclined only 20° above the horizontal to minimize bulk outflow. When a negative potential was applied to the pipette, radioactivity appeared in the saline and increased linearly with time for at least 20 min; without the current the release of radioactivity was minimal (Fig. 1). In a given time, for example, 10 min, the release was proportional to current up to at least 300 nA.

With 300 nA, ADP was released at about 2×10^{-14} mol/s. Such a current produced platelet thrombi in normal venules within 5 s. The micropipette tip is less than 5 μM from the vessel wall, so that the concentration of ADP *outside* the wall at least may be quite high.

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Interaction between anticonvulsant drugs and vitamin D

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