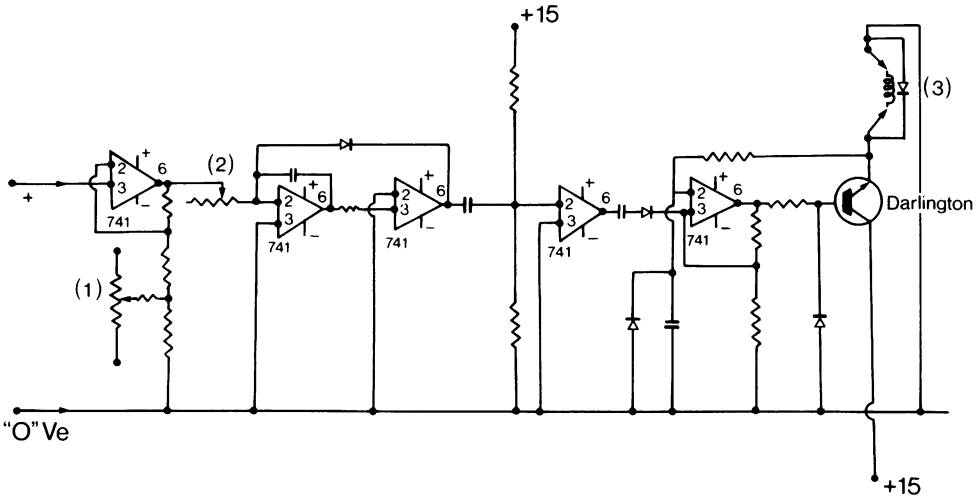


**Figure 1** 1. Set Zero Control. 2. Sensitivity control. 3. Coil of digital counter.

starts. The second is the sensitivity control which sets the counting rate. Both controls are adjusted with multi-turn potentiometers and ten-turn duodials. The Planimeter is at present used in the Department of Pharmacology, Royal College of Surgeons.

**References**

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## $^{31}$P and $^{1}$H n.m.r. studies of coenzyme binding to dihydrofolate reductase

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The coenzyme NADPH binds very tightly ($K_a > 10^8 M^{-1}$) to *L. casei* dihydrofolate reductase, and also substantially increases the affinity of the enzyme for inhibitors such as methotrexate. We have been studying the binding of coenzymes and coenzyme 'fragments' such as 2'AMP to the enzyme by $^{31}$P and $^{1}$H n.m.r. The 2'-phosphate group plays an important part in binding, and binds preferentially in the dianionic form. This preference is only 16-fold for 2' AMP, but over 1000-fold for NADP$^+$ and NADPH. Together with changes in the chemical shift of the 2'-phosphate $^{31}$P resonance, this indicates that the environment of the 2'-phosphate in the complex is significantly altered by the binding of the rest of the coenzyme. The binding constant of 2'AMP, for example, may not therefore be an accurate measure of the contribution to binding of this part of the whole coenzyme.

Although NADPH binds three orders of magnitude more tightly than NADP$^+$, the $^{31}$P and $^{1}$H n.m.r. spectra show that the mode of binding of the adenine, 2'-phosphate and pyrophosphate moieties is closely similar for oxidized and reduced coenzymes. The large differences in binding energy can thus be localized to a difference in the interactions of the nicotinamide ring itself. However, binding of reduced nicotinamide mononucleotide is too weak to be detectable. The effects of coenzyme binding on the $^{1}$H n.m.r. spectrum of the protein show that binding of NADP$^+$ and NADPH (in contrast to that of 2'-AMP) is accompanied by conformational changes which differ for the two forms of the coenzyme.

The effects of inhibitors such as methotrexate or trimethoprim on the bound coenzyme are also largely localized to the nicotinamide ring binding site – changes in the $^{31}$P chemical shifts are minimal, but there is a large change in the $^{13}$C chemical shift of [carboxamido-$^{13}$C]-NADP$^+$.