BIOCHEMISTRY

Direct Determination of Drug Concentrations in Biological Fluids by Polarography

It is an established fact that part of the initial testing of new drugs involves their estimation in biological fluids from animals or patients receiving the drug. This information is required to establish dosage regimes and to study rates and methods of elimination from the body. Hitherto, the use of physico-chemical techniques such as spectrophotometry, colorimetry and polarography has frequently required a separation of the drug from interfering materials present in the biological fluid. This communication suggests that, in suitable circumstances, the polarographic method can be successfully employed without any such separation.

During the clinical testing of the new anti-tubercular drug 2-ethyl-4-thiocarbamidopyridine (1314 *TH*) it became necessary to determine small concentrations (0·1–5·0 μgm./ml.) of the drug in serum and cerebrospinal fluid, and larger concentrations (>30 μgm./ml.) in urine from patients receiving the drug. Since 1314 *TH* gives a well-defined polarographic wave ($E_{1/2} = -0{\cdot}78$ volt vs. standard calomel electrode at *p*H 7·0) the utility of the polarographic method was investigated. (Full details of the electrochemistry of polarographic reduction of 1314 *TH* and of the methods described below for estimating its concentration in biological fluids will be the subject of further publications.)

It was found that, using a laboratory-constructed, pen-recording polarograph at its maximum sensitivity of 200 mm./μamp., de-gassed, normal sera of the rat, rabbit, horse and human were free from detectable polarographic waves over the potential range −0·1 to about −1·1 volts, using an internal silver wire anode. This potential range is conveniently referred to as the accessible region of potential. Normal rat and human urine were free from polarographic waves only over the region −0·2 to −0·7 volt. Moreover, the slope of the residual current and the oscillations due to growth and fall of drops were much greater than usual.

Accordingly, polarograms of known concentrations of 1314 *TH* in the above sera and cerebrospinal fluid were recorded and a well-defined wave of height proportional to concentration was obtained. There appeared to be no appreciable change of half-wave potential between serum solutions and aqueous solutions of the same *p*H. There was no need to add any extra supporting electrolyte or buffering agent beyond that already contained in the fluid. The limit of sensitivity with present equipment is ~0·2 μgm./ml. but it is hoped that this can be improved by suitable instrumentation. It was necessary to acidify the urine in order to bring the 1314 *TH* wave into the accessible region of potential, and addition of 5 per cent by volume of glacial acetic acid was a suitable procedure. The sensitivity was decreased to about 5 μgm./ml. because of the larger residual current. The polarographic method has been applied to serum, cerebrospinal fluid and urine from patients receiving 1314 *TH*.

Although most of the work on this problem has been devoted to the estimation of 1314 *TH* there seems no reason why any compound should not be estimated, the half-wave potential of which is in the accessible regions of potential and which does not interact significantly with serum proteins. Other compounds studied briefly in these laboratories to which the direct polarographic method can probably be applied are: pyrazine-2-carboxyamide, certain nitrofuran derivatives, *iso*nicotinylhydrazide and terramycin. The latter two drugs possess half-wave potentials close to the negative limit of accessible potential for serum, and some *p*H adjustment may be necessary to obtain highest sensitivities.

Since this preliminary communication was written, my attention has been directed to the work of Teisinger[1] on the direct polarographic determination of nitrobenzene in serum quoted by Brezina and Zuman[2]. It appears that the approach described above is not novel but has lain in abeyance for some twenty years.

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[1] Teisinger, J., *Casopis lékáru ceskych*, **76**, 325 (1937).
[2] Brezina, M., and Zuman, P., "Polarography in Medicine, Biochemistry and Pharmacy", 313 (Interscience, 1958).

Effect of Azaserine (o-Diazoacetyl-L-serine) on the Pyridine Nucleotide Levels of Mouse Liver

The amount of diphosphopyridine nucleotide in mouse liver has been shown to increase upon the intraperitoneal injection of a number of pyridine derivatives[1,2]; nicotinamide is the most effective, raising the level 8–10 times. Although nicotinic acid is as effective as many of the analogues, it appears to inhibit the synthesis when administered with nicotinamide, as compared with nicotinamide alone. Inhibition of synthesis from nicotinamide injection has also been reported with the administration of the purine antagonists 6-mercaptopurine and thioguanine[1,3]. These inhibitions appear to affect only the level of diphosphopyridine nucleotide attained from nicotinamide injection and the rate of its subsequent return to the normal level, but do not appear to affect the basal level normally found in the liver.

Recently, the injection of azaserine has been shown to inhibit markedly diphosphopyridine nucleotide synthesis resulting from nicotinamide injection[4]. Unlike the effects of the other inhibitors of this system, the administration of azaserine alone causes a marked reduction in the normal levels of the pyridine nucleotide.

We have found that subcutaneous injection of azaserine at a dosage of 200 mgm./kgm. rapidly reduces the level of pyridine nucleotide in the liver. The diphosphopyridine nucleotide-levels in the livers of 2–3 months old ($BALB/cAN \times DBA/2J$) F_1 hybrid mice were followed using the methylethyl ketone method previously described[1]. In Fig. 1, it can be seen that the pyridine nucleotide-level drops to a minimum value, which is about a nine-fold decrease from the level in control mice injected with 0·85 per cent saline, in 1–2 hr.

After reaching a minimum value, the pyridine nucleotide content was found to rise slowly over a 24-hr. period, approaching the level of the saline-injected, control animal. It appears that this dosage is somewhat toxic with approximately 25 per cent of the animals dying between 20 and 24 hr. after injection of azaserine.