The dye uptake, however, varies with protein concentration as well as from one protein to another, at least for the lowest and the highest concentrations. At the lowest concentrations, the different binding capacity of albumin and $\gamma$-globulin can most likely be ascribed to the chemical nature of the dye, whereas at the highest concentration, where the behaviour is reversed, another factor may be involved, namely the protein density on paper. This effect was previously observed in the case of bromophenol blue[2,3].

In addition to this behaviour of NIG with regard to different proteins, there are some other factors that must be considered. NIG does not satisfy the rest of the criteria of a dye suitable for routine work. Although it is cheap, easy to use and stable, it does not stain only protein, but paper as well. Furthermore, since it is a mixture of different dyes, its behaviour is subject to variation from one batch to another, a property it has in common with a great number of dyes currently used.

We are therefore of the opinion that NIG cannot be recommended as a stain for the quantitative determination of protein in paper electrophoresis.

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Received January 6th, 1958

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## Chromatographic evidence for the existence of a protactinate ion

So far no evidence for the existence of a protactinate seems to exist, except for the observation that the yield of $Pa_2O_5$ is decreased if this substance is washed with alkali.

We wish to present here some chromatographic and electrophoretic evidence that Pa(V) may be dissolved in alkali and that such a solution has similarities to a solution of tantalate. The isotope of Pa used in these experiments was a preparation of $^{233}Pa$ in 6 $N$ HCl which was shown in numerous experiments to be free from fluoride and radioactivities other than $^{233}Pa$. When the 6 $N$ HCl solution is evaporated and stirred with 5 $N$ NaOH, a suspension is obtained, which when placed on a paper strip and developed with $N$ NaOH stays at the point of origin (Fig. 1). Another sample

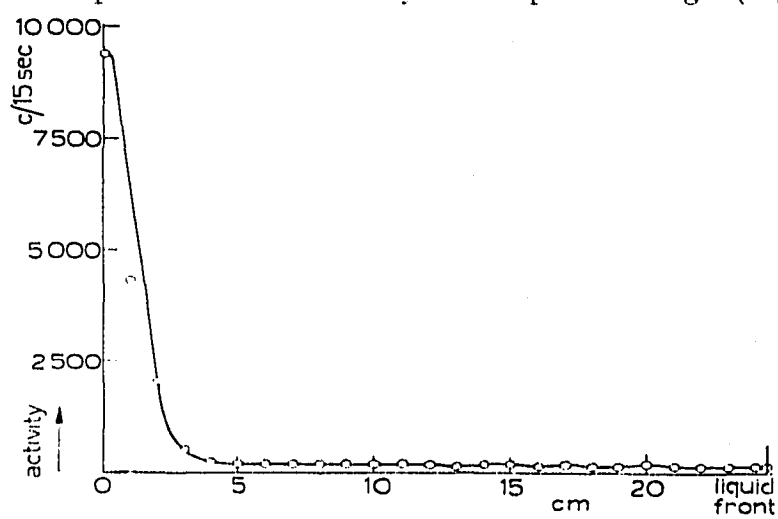


Fig. 1. Distribution of $^{233}Pa$ on the chromatogram when the tracer is mixed with 5 $N$ NaOH solution. Whatman No. 1 paper. Developing solvent $N$ NaOH.

was evaporated and fused with about 100 mg of NaOH in a micro-beaker for a few minutes and the melt dissolved in sufficient water to produce a 30% NaOH solution. When this solution is chromatographed with $N$ NaOH on a paper strip, part of the activity migrates with the solvent and part stays at the point of application (Fig. 2).

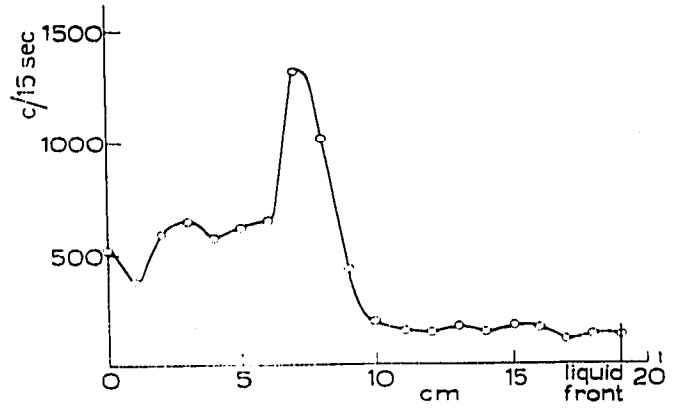


Fig. 2

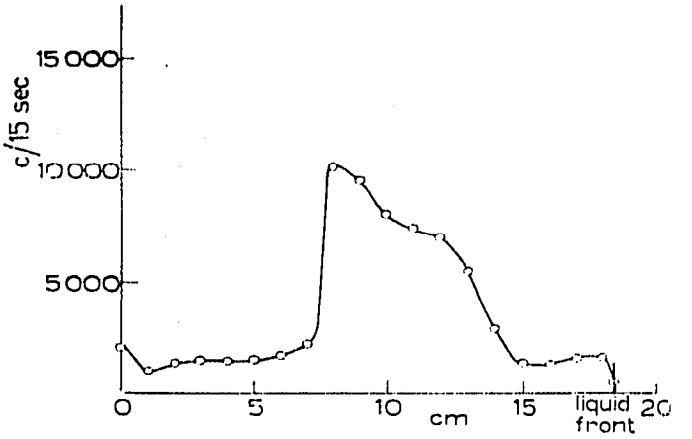


Fig. 3

Fig. 2. Chromatogram of a NaOH fusion of Pa tracer. Solvent: $N$ NaOH.
Fig. 3. Chromatogram of a KOH fusion of Pa tracer. Solvent: $N$ KOH.

According to SCHOELLER AND POWELL[1] Ta(V) dissolves in a NaOH melt but not in 30% NaOH, and is more soluble in a KOH melt than in NaOH. We thus evaporated another sample and fused it as above with 100 mg KOH. As shown in Fig. 3 the Pa(V) activity is almost completely in the region of $R_F$ 0.5 with only traces at the start. Since barium tantalate is insoluble, we added some $Ba(OH)_2$ to the KOH melt, applied the suspension so formed to paper and developed with saturated baryta. As shown in Fig. 4 the $^{233}Pa$ activity stays at the start under these conditions. Paper electrophoresis of the melt diluted with sufficient water to bring the concentration of KOH to about $N$ yielded a single slow moving anionic band as shown in Fig. 5. Paper chromatography indicates already that the soluble form of protactinium is more adsorbed than many anions, so the slow movement may be due to adsorption and/or a low charge on the anion. From past experience and comparison with the movement of $CrO_4^{--}$ we take it as most likely that the anion has one negative charge.

We have thus observed a form of Pa(V) soluble in alkali under conditions which render Ta(V) soluble and with properties similar to tantalate. Its movement in paper chromatography

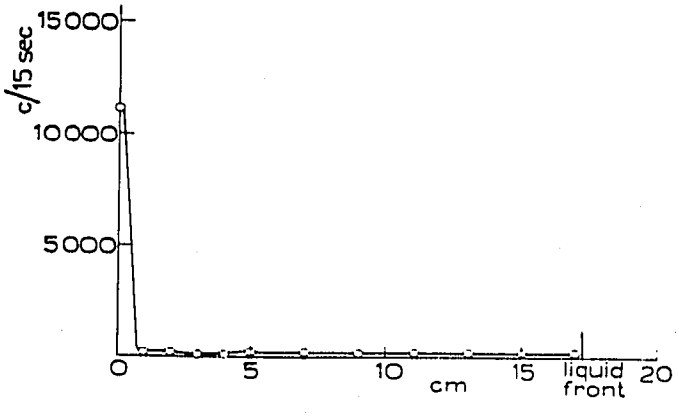


Fig. 4

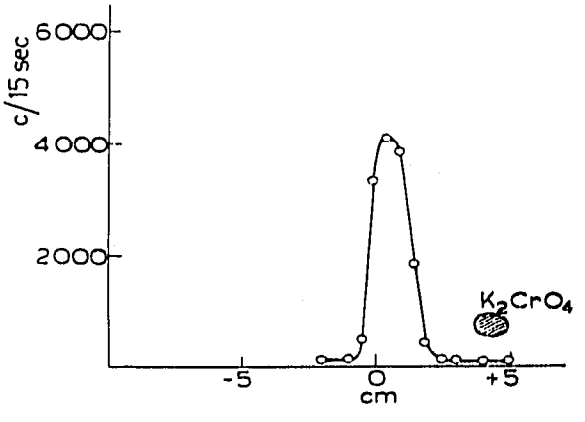


Fig. 5

Fig. 4. Chromatogram of a mixture of the KOH melt with $Ba(OH)_2$ solution. Solvent: saturated $Ba(OH)_2$.
Fig. 5. Electropherogram of the KOH melt of Pa tracer (diluted to $N$ KOH). 300 V, 1 h 23 min with $N$ KOH as electrolyte. A spot of $CrO_4^{--}$ run on the same sheet is also shown.

with aqueous solvents seems to exclude the possibility of a colloidal suspension. Its electrophoretic movement suggests the anionic nature of the Pa(V) in solution. We hence propose that a protactinate ion has been prepared and that contrary to current belief Pa(V) has amphoteric properties. The failure of other workers to obtain a soluble protactinate may be due to the fact that most experiments were conducted with macro amounts of $^{231}Pa$ which perhaps is not very easily attacked.

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Received March 7th, 1958

## Detection of pyrimidine deoxyribosides and deoxyribotides on paper chromatograms

Because of the biological significance of pyrimidine deoxyribosides and deoxyribotides, their detection on paper chromatograms is important. This can be achieved by spraying with the cysteine–sulphuric acid reagent[1] or by microbiological methods[2]. In the former method the colour fades quickly, while the latter method is time-consuming. The diphenylamine spray[3], which is very useful for purine deoxyribosides and deoxyribotides, is not very suitable for the pyrimidine derivatives owing to the stability of the glycosidic bond. The sensitivity of the method is therefore much less in the case of pyrimidine derivatives than in that of purine derivatives. It is well known that bromination[4] of the pyrimidine ring in nucleosides and nucleotides renders the glycosidic bond acid-labile. We have found that the diphenylamine method can be used to detect the pyrimidine nucleosides and nucleotides after treatment of the chromatogram with bromine. The method is as follows: The dried chromatogram is put on a glass plate and dabbed carefully with a piece of cotton-wool soaked in a solution of bromine–water–acetic acid (1:50:10 v/v/v). To prevent the zones from becoming diffuse, the paper must not be too wet. The paper is placed between two glass plates at 100° for 5 minutes and is then treated with the diphenylamine–sulphuric acid reagent in the standard manner[3]. Thymidine, deoxycytidine, deoxyuridine, thymidylic acid and deoxycytidylic acid all give blue spots on paper when submitted to this procedure. The sensitivity of the method is recorded in Table I, and that of the diphenylamine–sulphuric acid spray without bromination, is given for comparison.

TABLE I

The amounts recorded are those that give a distinctly visible spot on a chromatogram

| *Substance* | *Without bromination* | | *With bromination* | |
|---|---|---|---|---|
| | *μ moles* | *μg (approx.)* | *μmoles* | *μg (approx.)* |
| Deoxycytidylic acid | 1/4 | 72 | 1/16 | 18 |
| Deoxycytidine | 1/8 | 28 | 1/32 | 7 |
| Thymidylic acid | 1/4 | 80 | 1/16 | 20 |
| Thymidine | 1/4 | 60 | 1/16 | 15 |
| Deoxyuridine | 1/4 | 57 | 1/16 | 14 |

It can be seen from the table that bromination increases the sensitivity of the diphenylamine spray four times.

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Received March 24th, 1958