40% on the second day but the concentration was low, the estimate lying between 22 and 34 i.u./g.

3. The total vitamin D in the kidneys on the first and second days was estimated to be about 220 and 180 i.u., respectively. The corresponding concentrations were 270 and 200 i.u./g., which were of the same order as that found in the liver in previous experiments. It is suggested that the kidneys accumulate the vitamin by acting as a barrier to its elimination in the urine.

4. In lungs, spleen and adrenals, which contributed little to the total vitamin D content of the body, the concentration was of the order of 100 i.u./g.

5. From the results of the present experiment and those already published, there is no evidence that organs not examined (bones, etc.) contain any appreciable amounts of the vitamin.

We wish to thank Mr A. L. Bacharach, Glaxo Laboratories, Ltd., for the gift of ergocalciferol. We are indebted to Miss N. L. Gass and Mr S. R. Smith for their valuable technical help, and Miss J. Allen for computing assistance.

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Quantitative Estimation of Glucose by Paper Partition Chromatography

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(*Received* 18 *February* 1954)

In the course of recent work it became desirable to develop a simple method for the quantitative estimation of reducing carbohydrates on paper chromatograms, which would be more accurate than that of direct photometry (Gustafson, Sundman & Lindh, 1951) or spot area elution (McFarren, Brand & Rutowsky, 1951), but less time-consuming than elution followed by micro-estimation (Flood, Hirst & Jones, 1948).

It was found that the coloured product formed by heating aniline hydrogen phthalate with glucose on paper could be quantitatively eluted and its concentration determined colorimetrically. Blass, Macheboef & Numez (1950) attempted elution with various organic solvents, but found it incomplete. The technique described below was however found to give reproducible results.

EXPERIMENTAL

Anhydrous glucose was dried to constant weight at 60° *in vacuo*. Quantities equivalent to 50, 100, 150 and 200 μg. were applied to Whatman no. 4 paper using an 'Agla' micrometer syringe. The spots were air-dried and the chromatograms developed 18 hr. by the ascending technique in the following solvent system: *n*-propanol–ethyl acetate–water (7:1:2, v/v) (Baar & Bull, 1953). The chromatograms were then air-dried and drawn through a solution consisting of 85% (v/v) aqueous *iso*propanol (400 ml.), phthalic acid (6·64 g.) and aniline (3 ml.). Hopkin & Williams 'AnalaR' aniline was redistilled before use. The reagent was kept in a dark bottle in the refrigerator. Excess of the solution was removed from the papers by means of a rubber roller. The air-dried sheets were placed on the bottom shelf of an electric oven and heated for 15 min. at 115±1°. The stained areas were cut out as rectangles allowing a suitable margin and choosing a size suitable to all concentrations. One unstained area of similar dimensions was used to provide a solvent blank. Each of these areas was extracted with glacial acetic acid (4 ml.) at room temperature for 24 hr. The typical absorption curve was established on a Unicam Spectrophotometer (SP. 600) and is shown in Fig. 1. By choosing a wavelength of 480 mμ., a calibration graph was established. This

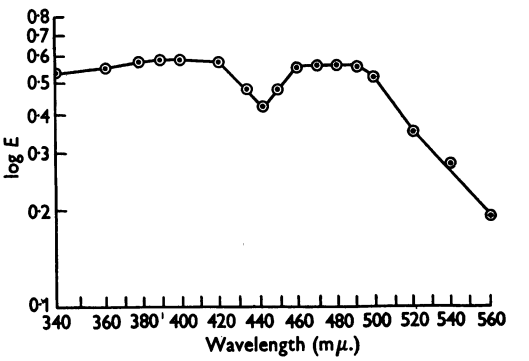

Fig. 1. Typical absorption curve of coloured product of reaction between glucose and aniline hydrogen phthalate. Colour developed on paper chromatogram and extracted with glacial acetic acid.

Table 1. *Factors influencing the colour yield*

Application area*	Reading	Time of heating (min.)	Reading	Extraction time† (hr.)	Reading	Stained area kept in dark (days)	Reading	Stained area kept in light (days)	Reading
1	0·238	5	0·223	0·25	0·081	1	0·239	1	0·240
2	0·242	10	0·240	0·5	0·100	2	0·241	2	0·236
3	0·240	15	0·239	2	0·176	3	0·238	3	0·221
		20	0·241	4	0·213	4	0·240	4	0·214
		25	0·239	6	0·220				
		30	0·235	8	0·230				
				12	0·241				
				24	0·240				
				2 days	0·238				
				5 days	0·221				
				7 days	0·214				

* Varied by increasing dilutions and represented as simple integers of the original volume.
† These results also indicate the stability of the extracted colour.

Table 2. *Recovery of glucose from chromatograms*

Glucose (μg.)	Recovery* (%)
25	102·0
50	99·8
100	100·0
150	97·0
200	98·5

* By comparison with glucose eluted and determined by the method of Jones & Pridham (1953). For details see Experimental section.

obeyed Beer-Lambert's law over a range of 0–300 μg. glucose with an optical density of 0·225 per 100 μg.

Various possible sources of error were investigated. These were influence of application area, time of heating, extraction time, ageing of colour reagent, different grades of paper and the stability of the colour complex in the presence or absence of light. Cells of 1 cm. depth were employed throughout.

To obtain a measure of the absolute accuracy of the partition chromatography employed, the above-described method was compared with an elution method, followed by micro-estimation. Two sheets were run in parallel, one stained and the corresponding areas cut from the other. The glucose was eluted by refluxing in a 'Quickfit' test tube containing 1 ml. of distilled water. The rolled paper was attached by means of a glass hook to the spiral inner coil tube of the condenser. The cold eluate was adjusted to 1 ml. and its glucose content estimated colorimetrically using benzidine (Jones & Pridham, 1953) at 290 mμ.

RESULTS

For a range of 50–200 μg. the coefficient of variation for single observations was found to be 0·72 %. This average was obtained from individual values of 0·57 % for 50 μg., 0·99 % for 100 μg., 0·71 % for 150 μg., and 0·68 % for 200 μg. The detailed results of the investigation of possible sources of error are shown in Table 1. Table 2 indicates the recoveries obtained by the new method in comparison with elution of glucose followed by micro-estimation.

Comparison of the mean values obtained using 'AnalaR' grade aniline manufactured by Hopkin & Williams and by British Drug Houses showed the following differences from the first mentioned: 50μg., +0·55%; 100μg., +0·02%; 200μg., +0·14%.

The effect of ageing of the colour reagent was investigated for a period of 2 weeks and the following results were obtained expressed as coefficients of variation for single observations. For 50 μg. the coefficient was 1·0 %, for 100 μg., 1·135 %, and for 200 μg., 0·35 %. Three runs were performed on Whatman no. 2 paper and compared with mean values obtained on no. 4 paper using Hopkin & Williams's aniline. No. 2 paper showed the following differences: for 50 μg., +0·75 %; for 100 μg., +0·55 %; for 200 μg., +0·44 %.

DISCUSSION

Although the above-described method is a general one, lacking specificity, it is well suited to carbohydrate estimation on chromatograms, since the lack of specificity is replaced by chromatographic separation. The high sensitivity of the method makes it possible to estimate very small quantities of glucose on a chromatogram employing a simple and reproducible technique.

SUMMARY

1. A simple colorimetric technique for the quantiative estimation of glucose eluted from chromatograms has been developed.

2. The results of investigating various sources of error are shown.

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