thioacetamide on the liver cell—by determining an intracellular accumulation of calcium ions—played any part in the action of dimethylnitrosamine.

SUMMARY

1. The acute hepatotoxic action of dimethylnitrosamine was investigated with homogenates and mitochondrial preparations from the livers of rats injected with 100 mg./kg. body weight at various times between 8 and 24 hr. before killing.

2. Aerobic oxidation of pyruvate, octanoate, L-malate, citrate, L-glutamate, α-oxoglutarate and β-hydroxybutyrate progressively decreased after the twelfth hour and was grossly reduced by the twentieth hour. Aerobic oxidation of choline was only slightly reduced. Succinoxidase activity and anaerobic glycolysis were unaffected even when necrosis was extensive.

3. Oxygen uptakes of the diphosphopyridine nucleotide-linked systems could be greatly raised by the addition of diphosphopyridine nucleotide to the mitochondrial incubation medium. No other co-factor tested was effective.

4. Dimethylnitrosamine did not show an effect *in vitro* on normal rat-liver homogenate. Ethylenediaminetetra-acetic acid did not reverse the inhibition of aerobic oxidation of mitochondrial preparations made 16 hr. after poisoning.

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Identification of Amino Sugars

By M. J. CRUMPTON*
Microbiological Research Establishment, Porton, Wilts

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D-Glucosamine and D-galactosamine have long been known to occur naturally. During the last few years, however, D-gulosamine (van Tamelen, Dyer, Carter, Pierce & Daniels, 1956), D-mannosamine (Comb & Roseman, 1958), D-fucosamine (Crumpton & Davies, 1958), 'muramic acid' (3-*O*-α-carboxyethyl-D-glucosamine; Strange & Kent, 1959) and an *N*-acetylaminohexuronic acid (Clark, McLaughlin & Webster, 1958) have been isolated in crystalline form from natural products.

Amino sugars occur in *N*-acetylated form in those bacterial polysaccharides which have been investigated, but since acid hydrolysis is usually the first step in analysis the unacetylated sugar is released. The simplest method of detection and estimation is that described by Elson & Morgan (1933), in which neutralized hydrolysates containing 2-amino-2-deoxy aldose sugars give a characteristic chromophore on treatment with alkaline acetylacetone and *p*-dimethylaminobenzaldehyde hydrochloride. A variety of modifications of this method have been described (see Rondle & Morgan, 1955*a*) but, although the wavelength of maximum absorption may differ with different modifications, for a particular technique the majority of sugars give the same absorption maximum.

* Present address: Laboratory of Cellular Physiology and Metabolism, National Heart Institute, Bethesda 14, Md., U.S.A.

Paper chromatography (Partridge, 1948) has been widely used for provisional identification. Although it is possible by this means to differentiate a number of these sugars, some of them occupy overlapping positions. Leaback & Walker (1957) have also shown that owing to ionic interactions between amino sugar salts and weakly basic or acidic chromatographic solvents pure sugars may give more than one spot. It is therefore apparent that even a provisional identification by this means is unreliable. Nevertheless, in many cases, amino sugars present in natural materials have been provisionally identified by paper chromatography alone, authentic samples of only glucosamine and galactosamine being used for reference. In some cases this tentative identification has been supported by the characterization of the aldose formed on degradation of the sugar with ninhydrin (Stoffyn & Jeanloz, 1954); this method, however, distinguishes only epimeric pairs of sugars. In relatively few cases have the sugars been isolated in crystalline form and unequivocally identified by comparison of their properties with those of authentic samples. This is laborious and requires relatively large amounts of material and there is need for an alternative and more rapid method of identification.

The identification of an amino sugar by measurement of the volume of acid required to elute it from a column of Dowex 50 resin has been suggested by Gardell (1953). A method such as this would be especially valuable in being applicable to small amounts of material and would, in certain cases, permit the isolation of the amino sugar for confirmation of its identity by other means.

This paper describes the order in which a number of amino sugars are eluted with acid from columns of Zeo-Karb 225 resin and includes some data on the estimation of these sugars and the orders of separation of the *N*-acetylated sugars on paper chromatograms and paper electrophoresis. It is possible from the data presented to make a more accurate judgement of the identity of certain sugars, without using the authentic sugar as a standard for comparison, than by paper chromatography alone. Rigorous identification is still, however, possible only after the crystalline sugar has been isolated. A preliminary report of this work has been published previously (Crumpton, 1958).

MATERIALS AND METHODS

Amino sugars. D-Glucosamine and *N*-acetyl-D-glucosamine were commercial samples (L. Light and Co. Ltd., Bucks). The preparation of talosamine described by Crumpton (1957) was used in some experiments and D-fucosamine hydrochloride was prepared according to Crumpton & Davies (1958). D-Fructosamine acetate (1-amino-1-deoxy-D-fructose) was synthesized by the method of Maurer & Schiedt (1935). Grateful acknowledgement is made for the following gifts: D-mannosamine and D-talosamine hydrochlorides (Kuhn & Bister, 1957; Kuhn & Fischer, 1958), D-gulosamine hydrochloride (van Tamelen *et al.* 1956), D-glucosaminuronic acid (Heyns & Paulsen, 1955), natural and synthetic muramic acid and a synthetic stereoisomer (Strange & Kent, 1959), *N*-acetyl-D-xylosamine (Wolfrom & Anno, 1953), *N*-acetyl-D-allosamine (Jeanloz, 1957), and D-galactosamine hydrochloride and *N*-acetyl-D-galactosamine (Professor W. T. J. Morgan, F.R.S., The Lister Institute, London, S.W. 1).

Aqueous solutions of the amino sugar hydrochlorides (0·1%, calc. as free base) were stored at −10°. *N*-Acetyl-D-xylosamine and *N*-acetyl-D-allosamine were deacetylated by hydrolysis with 0·5N-HCl in sealed ampoules at 100° for 5 hr. The hydrolysates were evaporated to dryness *in vacuo* and redissolved in water to give 0·1% soln. as free base; 100% recoveries of amino sugar were assumed when calculating the volume of water to be added. *N*-Acetyl derivatives of other amino sugars were prepared as described by Roseman & Ludowieg (1954). To 1 ml. of a 0·2% soln. of the amino sugar was added 1·5 ml. of 0·5% $NaHCO_3$ soln. and immediately afterwards 2 drops of A.R. acetic anhydride. The mixture was shaken and allowed to stand at room temperature for 24 hr.; Na^+ was removed by passage through a column of Zeo-Karb 225 resin (The Permutit Co. Ltd., London, W. 4; H^+ form washed with water to pH 7) and the neutral eluate containing the *N*-acetyl amino sugar was freeze-dried. Unchanged amino sugar could not be detected in N-HCl eluates of the column collected after elution of the *N*-acetyl amino sugar. Recoveries of the *N*-acetylated sugars were therefore taken to be 100% and the sugars were redissolved in water to give 0·1% solutions.

Amino sugar determination. Amino sugars were estimated by the method of Rondle & Morgan (1955*a*) with 20–100 μg. amounts of base. Colour intensities were measured in a Unicam SP. 600 spectrophotometer with a 2 cm. cell at the wavelength of maximum absorption. Absorptions of amino sugars other than muramic acid and fructosamine were determined at 530 mμ after 1–2 hr. and are expressed as a percentage of that given by an equal weight of glucosamine base. Colour intensities given by muramic acid were measured at 505 mμ after 24 hr. and of fructosamine at 550 mμ after 1–2 hr.

Partition chromatography. Single-dimensional descending paper chromatography was used with Whatman no. 1 paper and the following solvent systems: phenol–aq. NH_3 soln. (Dent, 1948), butanol–acetic acid–water (4:1:5, by vol.) and butanol–pyridine–water (6:4:3, by vol.). Sugars were applied in 100 μg. amounts in volumes of 10 μl. Papers were sprayed with alkaline acetylacetone followed by the *p*-dimethylaminobenzaldehyde hydrochloride reagent (Partridge, 1948) to reveal amino sugars and their *N*-acetyl derivatives. The rate of movement of each sugar has been expressed relative to that of glucose (R_G) determined on the same chromatogram.

Paper electrophoresis. Electrophoresis was carried out in borate buffer, pH 10 (sodium tetraborate, 23·4 g., and N-NaOH, 30 ml./l.), on Whatman no. 3 paper by the enclosed-strip method (Foster, 1952). The applied p.d. was 1300 v, which gave a current not greater than 25 mA, and the duration of electrophoresis was 2·5 hr. *N*-Acetyl amino

sugars were revealed by spraying with *p*-anisidine–HCl (Hough, Jones & Wadman, 1950) and their rates of movement have been expressed relative to that of glucose run at the same time (R_M).

Ion-exchange resin chromatography. Amino sugars were examined according to the method of Gardell (1953), as described by Rondle & Morgan (1955*b*), with a column filling of Zeo-Karb 225 resin (8% cross-linkage; > 200 mesh particle size; H^+ form) and 0·33 N-HCl as eluent. Glass columns about 43 cm. long and 0·8 cm. in internal diam. were packed with resin suspended in 4 N-HCl after two cycles through the Na^+ form and thoroughly washed with 0·33 N-HCl. Samples (200 μg. in 0·2 ml.) of the sugars were added and eluted with 0·33 N-HCl at a rate of 2 ml./hr.; 1 ml. fractions were collected in 10 ml. graduated test tubes with a photoelectric drop counter and a Locarte Fraction Collector (The Locarte Co., London, S.W. 7). The normality of HCl collected before and after elution of the amino sugars was checked by titration against standard alkali. The fractions were neutralized (phenolphthalein) by careful addition of N-NaOH, immediately back-titrated with 0·33 N-HCl until the indicator colour just disappeared and diluted to 2 ml. with water. The amount of eluted amino sugar present was determined as previously described and the absorption spectrum produced by that fraction containing the greatest amount of sugar was measured. Amino sugars other than muramic acid and fructosamine were estimated with glucosamine standards; amounts of muramic acid and fructosamine were measured with standards of the same sugar. Recoveries of 90–95% of the amount of amino sugar added to the column were usually obtained. The rate of movement of a sugar, measured in terms of the total effluent volume corresponding to the 1 ml. fraction which contains the greatest amount of sugar, has been expressed relative to that of glucosamine determined at the same time ($R_{glucosamine}$). Columns were regenerated after each run by elution with 4 N-HCl followed by 0·33 N-HCl until the effluent was 0·33 N.

RESULTS

Paper chromatography and electrophoresis

Although R values are not sufficiently reliable for the identification of sugars on paper chromatograms, the orders of separation of sugars are reproducible and when this order is known for a variety of solvent systems an idea of the identity of an unknown sugar may be gained without reference to authentic samples (Davies, 1957). In the present work the R_G value of each amino sugar was found to be reproducible within narrow limits for the same chromatogram, but comparison of the results of different chromatograms indicated that this value may vary by as much as 10% for sugars whose rate of movement is much slower than that of glucose. The orders of separation of the amino sugars and *N*-acetyl amino sugars which have been available for comparison on chromatograms developed in the solvents listed in the Methods section are shown in Table 1. The R_G values (average of two or more determinations; ±0·04) are given only to indicate the region occupied by a particular sugar and the degree of overlap to be expected between sugars which occupy adjacent positions. In butanol–pyridine–water and phenol–aq. NH_3 soln. all sugars gave a single spot. However, when butanol–acetic acid–water was employed as solvent amino sugars other than muramic acid and glucosaminuronic acid gave two overlapping spots. This was probably due to the formation of some amino sugar acetate by interaction of the amino sugar hydrochlorides with the solvent (Leaback & Walker, 1957). For this reason R_G values for the amino sugar hydrochlorides in this solvent are not included in Table 1.

Amino sugars other than muramic acid and fructosamine gave cherry-red spots when sprayed with the hexosamine reagents; muramic acid gave a salmon-pink and fructosamine a reddish purple. *N*-Acetyl amino sugars other than *N*-acetylfructosamine were detected with the hexosamine reagents as purple spots or with *p*-anisidine–HCl, which gave different coloured spots with the different classes of sugars; *N*-acetylhexosamines produced light brown, *N*-acetylfucosamine yellow and *N*-acetylxylosamine reddish brown. No detectable reaction was given by *N*-acetylfructosamine with either of these spray reagents; the position of this sugar was revealed with either urea–phosphoric acid (Wise, Dimler, Davis & Rist, 1955) or orcinol–trichloroacetic acid (Klevstrand & Nordal, 1950).

The order of separation and R_M (±0·02) of the *N*-acetyl amino sugars on paper electrophoresis are shown in Table 2.

Detection and estimation of amino sugars

Samples (1 ml.) of each amino sugar at concentrations of 20–100 μg./ml. were treated with alkaline acetylacetone followed by the *p*-dimethylaminobenzaldehyde hydrochloride reagent, and the absorption spectra of the chromophores formed were measured 5 min., 6 hr. and 24 hr. after heating at 70° for 10 min. All of the sugars examined, except fructosamine and muramic acid, gave absorption spectra which were similar to the spectrum of glucosamine, with a peak of 530 mμ. Comparison of the absorption spectrum of talosamine with that of glucosamine showed, however, that the maximum absorption for talosamine coincides with a slightly higher wavelength of about 535 mμ. Fructosamine differed from other amino sugars in that the maximum absorption of its chromophore coincided with a wavelength of 550 mμ. In each case the wavelength of maximum absorption did not alter during the 24 hr. period of examination, although a slow diminution in the colour intensity at this wavelength was observed (cf. Rondle & Morgan, 1955*a*). The results obtained with muramic acid are shown in Fig. 1. After

Table 1. *Orders of separation of amino sugars and* N-*acetyl amino sugars on paper chromatograms*

R_G	Butanol–pyridine–water	R_G	Phenol–aq. NH_3 soln.	R_G	Butanol–acetic acid–water
0·23	D-Glucosaminuronic acid	0·61	D-Glucosaminuronic acid	0·68	D-Glucosaminuronic acid
0·62	D-Fructosamine	1·26	Muramic acid	—	D-Galactosamine
0·66	D-Allosamine	1·53	D-Glucosamine	—	D-Allosamine
0·69	D-Galactosamine	1·61	D-Galactosamine, D-Gulosamine, D-Mannosamine	—	D-Glucosamine, D-Talosamine, D-Gulosamine
0·75	D-Talosamine			—	D-Fructosamine
0·76	D-Glucosamine, Muramic acid	1·65	D-Allosamine	—	D-Mannosamine
0·77	D-Gulosamine	1·66	D-Talosamine	—	—
0·80	D-Mannosamine	1·68	D-Xylosamine	1·00	D-Xylosamine, D-Fucosamine
1·00	D-Xylosamine, D-Fucosamine	1·71	D-Fructosamine	—	D-Fucosamine
1·21	*N*-Acetyl-D-galactosamine	2·00	D-Fucosamine	—	Muramic acid
1·30	*N*-Acetyl-D-glucosamine	1·70	*N*-Acetyl-D-glucosamine	1·39	*N*-Acetyl-D-galactosamine
1·32	*N*-Acetyl-D-allosamine	1·72	*N*-Acetyl-D-mannosamine	1·38	*N*-Acetyl-D-glucosamine
1·36	*N*-Acetyl-D-mannosamine	1·81	*N*-Acetyl-D-gulosamine	1·44	*N*-Acetyl-D-allosamine
1·42	*N*-Acetyl-D-gulosamine	1·82	*N*-Acetyl-D-galactosamine	1·54	*N*-Acetyl-D-mannosamine
1·44	*N*-Acetyl-D-fructosamine	1·83	*N*-Acetyl-D-allosamine	1·62	*N*-Acetyl-D-talosamine
1·45	*N*-Acetyl-D-talosamine	1·84	*N*-Acetyl-D-xylosamine	1·65	*N*-Acetyl-D-xylosamine
1·61	*N*-Acetyl-D-fucosamine	1·88	*N*-Acetyl-D-talosamine	2·14	*N*-Acetyl-D-fucosamine
1·62	*N*-Acetyl-D-xylosamine	2·07	*N*-Acetyl-D-fucosamine	2·16	—
		—	—	—	—

Table 2. *Order of separation of* N-*acetyl amino sugars on paper electrophoresis*

R_M	
0·14	*N*-Acetyl-D-fucosamine
0·23	*N*-Acetyl-D-glucosamine, *N*-Acetyl-D-xylosamine
0·33	*N*-Acetyl-D-galactosamine
0·42	*N*-Acetyl-D-allosamine
0·60	*N*-Acetyl-D-talosamine
0·63	*N*-Acetyl-D-gulosamine
0·65	*N*-Acetyl-D-mannosamine

5 min. the maximum absorption occurred at 510 mμ, which changed on standing to 505 mμ. The colour intensity at the wavelength of maximum absorption increased markedly with time; absorptions at 6 and 24 hr. were about 150 and 230% respectively of the amount of colour measured after 5 min. For this reason muramic acid was estimated by measurement of colour intensities at 505 mμ after 24 hr. It can, however, be seen from Fig. 1 that the colour intensity at the wavelength of maximum absorption for glucosamine (530 mμ) does not alter during the test period of 24 hr. and thus muramic acid can be estimated as glucosamine at this wavelength. The amount of colour formed by muramic acid at 530 mμ was equivalent to 27·1 ± 1·6% (average of seven determinations) of that produced by an equal weight of glucosamine base.

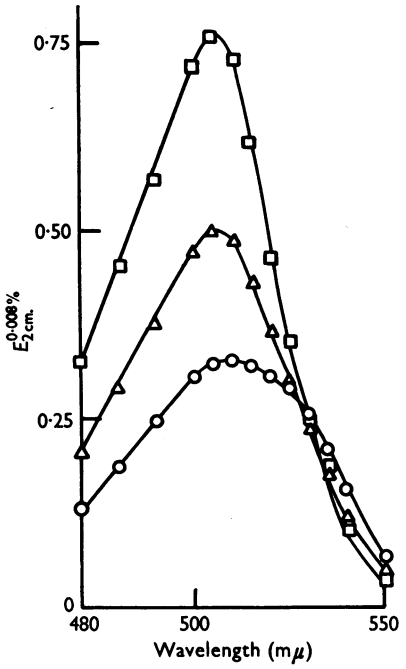

Fig. 1. Absorption spectrum of the chromophore formed by muramic acid. Determinations were made at: ○, 5 min.; △, 6 hr.; □, 24 hr.

Amounts of colour formed by equal weights of other amino sugars (μg. as base) were measured at 530 mμ and have been expressed relative to that produced at the same wavelength by an equal weight of glucosamine base. The 'equivalent glucosamine values' obtained are given in Table 3. It can be seen that equal amounts of different sugars produce different colour intensities at the wavelength of maximum absorption. The extinction values given by 20–100 μg. amounts of each sugar followed a straight-line relationship with the weight of sugar, and duplicate estimations carried out at different times on the same sample rarely differed by more than ± 3% for sugars other than fructosamine. Three samples of D-galactosamine were examined and the value quoted (92%) was

Table 3. *Amount of colour formed by amino sugars at 530 mμ relative to that produced by an equal weight of glucosamine*

	Equivalent glucosamine value (%±3%)
D-Glucosamine	100
D-Mannosamine	69
D-Gulosamine	81
D-Allosamine	83
D-Fucosamine	92
D-Galactosamine	92
D-Glucosaminuronic acid	100
D-Talosamine	112
D-Xylosamine	156

obtained with only one sample; the others gave less colour than this. Equivalent glucosamine values determined for fructosamine at a wavelength of 530 mμ varied from 105 to 158%, although the majority of values were between 126 and 131%. The factor responsible for this variation is not known, but for this reason fructosamine was estimated by comparison of colour intensities measured at the wavelength of maximum absorption (550 mμ) with those produced by known amounts of the sugar.

Ion-exchange resin chromatography

The effect of the strength of acid used for elution on the separation of amino sugars on columns of Zeo-Karb 225 resin was determined. Samples (0·4 ml.) containing 200 μg. each of glucosamine and galactosamine were eluted from the same column with hydrochloric acid of different strengths, within the range 0·2–0·9N. Only with 0·33N-acid did the sugars appear in completely separate peaks; acid of lower strength caused the peaks to broaden with consequent overlapping, owing to a slower elution rate, and acid of normality greater than 0·33N gave sharper peaks which also overlapped owing to elution of the sugars in smaller volumes of total effluent. The effect of different acid strengths on the peak positions of glucosamine and galactosamine, expressed as the total effluent volume (ml.) corresponding to the 1 ml. fraction containing the greatest amount of sugar, are shown in Fig. 2. Although the volume of effluent separating the peak positions increases as the normality of acid used for elution decreases, the ratio of the peak position of galactosamine to that of glucosamine ($R_{glucosamine}$) changes from 1·21 with 0·2N to 1·17 with 0·9N-hydrochloric acid and is constant at 1·20 within an experimental error of ±0·02, when acid of normality between 0·20 and 0·55N is used. Furthermore, the same average $R_{glucosamine}$ value for galactosamine was obtained with different columns containing varying amounts of resin and 0·33N-acid as eluent. It is concluded that this value is a characteristic property of galactosamine.

The $R_{glucosamine}$ values of the other amino sugars available for comparison were determined. Samples (0·2 ml.; 0·1%) of each sugar alone and together with 200 μg. of glucosamine were eluted from columns of Zeo-Karb 225 resin with 0·33N-hydrochloric acid and the peak positions in terms of total effluent volume (ml.) measured. The columns used had been previously standardized with a mixture of D-glucosamine and D-galactosamine (200 μg. of each sugar), which emerged in peak positions that were characteristic for a particular column provided that acid of the same normality was used as eluent. The composite results of the elution experiments are shown in Fig. 3; in each experiment only one sugar was run at a time together with glucosamine. The peaks of D-glucosamine and D-allosamine are omitted from this figure for the sake of clarity, since these and that of D-galactosamine were subject to considerable overlap. The absorption spectrum produced on treatment of the 1 ml. fraction containing the greatest amount of sugar with alkaline acetylacetone and *p*-dimethylaminobenzaldehyde hydrochloride was measured, since in this way it was possible to differentiate the peaks due to muramic acid and fructosamine from those of other amino sugars.

DISCUSSION

The presence of an amino sugar in a polysaccharide can most easily be demonstrated by treatment of an acid hydrolysate, after neutralization, with alkaline acetylacetone and Ehrlich's reagent; interference due to mixtures of simple carbohydrates and amino compounds should, however, be allowed for (Immers & Vasseur, 1952). Under the conditions of the Rondle & Morgan (1955*a*) method amino sugars other than fructosamine and muramic acid gave similar absorption spectra with a peak near 530 mμ. Since the chromophores formed by fructosamine and muramic acid possess characteristic absorption spectra, it is possible to detect these sugars, when present either alone or with other amino sugars, by this means. Muramic acid, unlike the other sugars, gave a maximum absorption and a maximum colour intensity which varied with time of standing, although the colour intensity at 530 mμ was independent of time during a test period of 24 hr. This sugar can therefore be estimated at 530 mμ in terms of glucosamine. A much more sensitive method, however, is to compare colour intensities measured at 505 mμ after 24 hr. with those given by known weights of the authentic sugar. The estimation of muramic acid has been previously described by Strange & Dark (1956), who used the method of Immers & Vasseur (1950). It was reported that the sugar

gave an absorption peak at 520 mμ and a colour intensity equivalent to 69% of that formed by an equal weight of glucosamine at the same wavelength. Comparison of these results with those obtained in the present work suggest that small differences in the method of estimation can result in significant changes in the absorption spectrum and in the amount of colour formed at the wavelength of maximum absorption. Equal weights of other 2-amino-2-deoxy aldoses produced different amounts of colour at 530 mμ and thus the Rondle & Morgan (1955*a*) method can be used only to estimate the amount of amino sugar present in a polysaccharide when the identity of the sugar is known. Mannosamine has been reported (Comb & Roseman, 1958) to give 81–84% of the colour formed by an equal weight of glucosamine, whereas a figure of 69% was obtained in the current work. This difference is presumably due to small variations in the method of estimation. Although Roseman & Daffner (1956) and other workers have reported that glucosamine and galactosamine yield equal amounts of colour, samples of galactosamine examined in the present work always produced less colour than the same weight of glucosamine. This difference may have been due to traces of impurities in the galactosamine samples. However, it is possible that galactosamine resembles the other

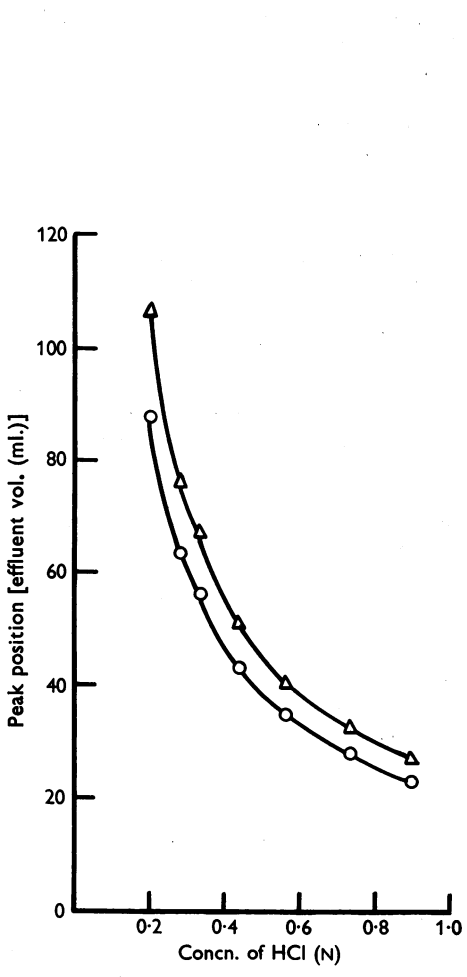

Fig. 2

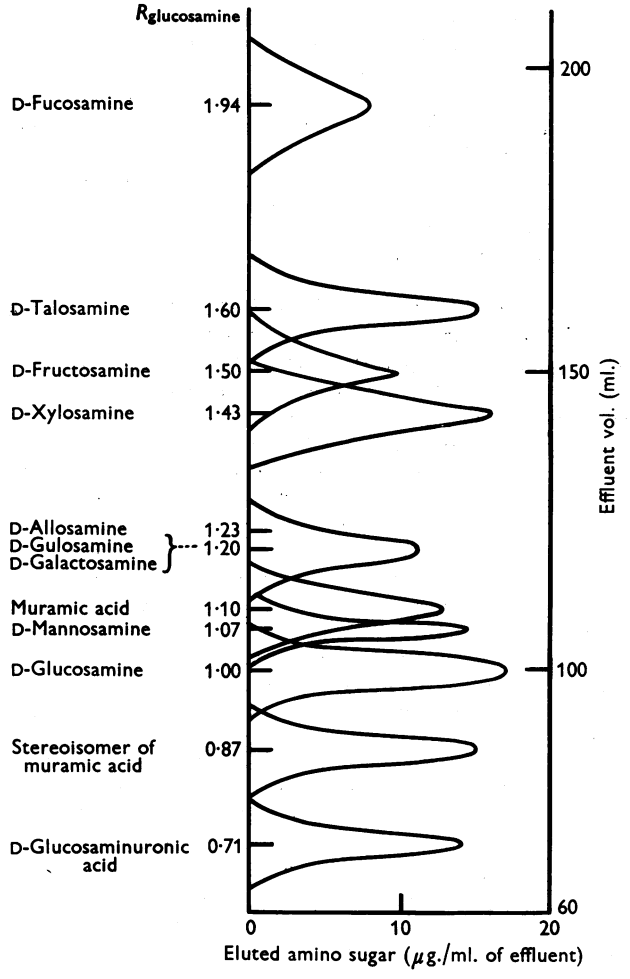

Fig. 3

Fig. 2. Relationship of normality of HCl used for elution of a Zeo-Karb 225 resin column to the peak positions (total effluent volume) of D-glucosamine (○) and D-galactosamine (△).

Fig. 3. Order of separation and $R_{glucosamine}$ values of amino sugars eluted from a column of Zeo-Karb 225 resin with 0·33 N-HCl.

hexosamines which have been examined and does not give the same colour intensity as an equal weight of glucosamine under the conditions of the Rondle & Morgan method. Further work will be necessary before this problem can be resolved. Differences in experimental technique may account for the results obtained by previous workers, since equal weights of fucosamine and glucosamine produced the same amount of colour when a longer period of heating with alkaline acetylacetone (30–35 min.) was employed, compared with an equivalent glucosamine value for fucosamine of 92% after heating for 20 min. (unpublished observations).

It is evident from a consideration of data obtained from the examination of amino sugars on paper chromatograms developed in a number of different solvent systems that it is not possible to identify an unknown sugar by this means. The method of identification suggested by Gardell (1953), in which the volume of acid required to elute the sugar from a column of Dowex 50 resin is measured, suffers from the disadvantages that acid of the same normality, together with a particular column which has been previously standardized with a sample of the authentic sugar, must be used. If, however, the peak position of an amino sugar is expressed relative to that of glucosamine run at the same time, then the resulting ratio is independent of the column dimensions, the amount of resin and the normality of the acid used for elution within the range 0·20–0·55N. $R_{glucosamine}$ values determined on separate occasions for any one amino sugar have rarely differed by greater than $\pm 0{\cdot}02$ and have usually agreed within $\pm 0{\cdot}01$. Since the majority of sugars have different $R_{glucosamine}$ values they can be identified by this means. Of the sugars that have been investigated only D-galactosamine, D-gulosamine and D-allosamine could not be distinguished by this technique. D-Mannosamine and muramic acid, which also occupied closely similar positions, could, however, be differentiated by examination of the absorption spectrum formed in the Rondle & Morgan test. It is evident from the results that certain amino sugars can also be isolated by this method. In these cases the identification of the sugar could be confirmed by paper-chromatographic and -electrophoretic examinations of its *N*-acetyl derivative. In this way it is possible to make a confident judgement of the identity of an unknown sugar.

As observed by previous workers, 0·33N-hydrochloric acid was found to give the most satisfactory separation of amino sugars with columns of Zeo-Karb 225 resin of the dimensions given in the Methods section. Under these conditions D-mannosamine does not separate completely from D-glucosamine (cf. Comb & Roseman, 1958) and the peaks of D-talosamine, D-fructosamine and D-xylosamine overlap. By careful selection of fractions it should nevertheless be possible to separate each of these sugars in a pure form. Neither muramic acid and D-mannosamine nor D-galactosamine, D-gulosamine and D-allosamine could, however, be separated by this technique.

Since this paper was submitted for publication Kuhn, Bister & Fischer (1958) have published *R* values for eight hexosamines and their *N*-acetyl derivatives on paper chromatograms.

SUMMARY

1. The volumes of 0·33N-hydrochloric acid required to elute a variety of amino sugars from a column of Zeo-Karb 225 resin have been measured and expressed relative to the volume required for glucosamine so that the values obtained can be used to gain an idea of the identity of an unknown sugar without reference to authentic samples.

2. The orders of separation have been recorded for the amino sugars and their *N*-acetyl derivatives on paper chromatograms developed in three different solvent systems and for the *N*-acetyl amino sugars on paper electrophoresis.

3. The absorption spectra formed by the sugars in the Rondle & Morgan (1955*a*) test have been compared and the amounts of colour produced by equal weights have been determined relative to the spectrum formed by glucosamine.

4. The data presented allow a number of amino sugars to be identified with confidence.

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The Relation between the Activity of a Lecithinase and the Electrophoretic Charge of the Substrate

By A. D. BANGHAM and R. M. C. DAWSON
Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge

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Fairbairn (1948) showed that the mycelium of the mould *Penicillium notatum* was a rich source of the enzyme phospholipase B which hydrolysed lysophosphoglycerides but not the parent diacylated compounds, e.g. lecithin, phosphatidylethanolamine. Dawson (1957) observed that preparations of this enzyme, although unable to hydrolyse pure lecithin, were able to attack the lecithin of the mixed phospholipid fractions isolated from liver. It was shown further that this property was due to the presence in the fractions of small quantities of monophosphoinositide and a polyglycerol phospholipid (Dawson, 1958*a*). When either of these substances was added to pure lecithin, the phospholipase B enzyme preparation rapidly hydrolysed the substrate with the formation of fatty acids and glycerylphosphorylcholine.

An examination of other lipid and non-lipid substances showed that only cardiolipin, a phospholipid structurally related to liver polyglycerol phospholipid, and the triglycerides tristearin and tripalmitin produced any appreciable activation of the enzymic attack upon lecithin. It was also observed that the addition of either monophosphoinositide or cardiolipin to lecithin caused a marked reduction in the extent to which the lecithin was extracted from its aqueous emulsion by organic solvents, e.g. ether and chloroform. This reduction in extractability of the lecithin ran closely parallel to the degree of activation of the enzyme system and, moreover, it was found that both the extractability change and the activation could be reversed by the addition of Ca^{2+} ions. It was therefore suggested that the activation was connected with some alteration in the surface properties of the lecithin particles (Dawson, 1958*b*). It was thought that the most likely change would be found in the ionizable groups contributing to the electric double layer between lecithin particles and the bulk aqueous phase. It was decided therefore to examine the particles by microelectrophoresis to determine the sign and magnitude of their mobilities, a measure directly related to their ζ potentials.

Preliminary results showed that the naturally occurring activators (monophosphoinositide and cardiolipin), when added to pure lecithin, produced a net negative charge on the surface of the mixed lipid when emulsified in buffer at the pH optimum of the enzyme. A further study of this phenomenon showed that other anionic amphipathic molecules (substances having a hydrophobic portion and a polar group: *amphi*, dual; *pathic*, sympathy; Hartley, 1936) could be used as activators, but that always a minimum net charge was a prerequisite for the development of enzymic attack on the emulsified substrate irrespective of the anionic species tested. The inhibitory effect of various