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The Acute Toxic Action of Dimethylnitrosamine on Liver Cells

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Dimethylnitrosamine has been shown to be selectively hepatotoxic for several species of mammals, producing demonstrable necrosis within 24 hr. (Barnes & Magee, 1954) and, on prolonged administration, to produce malignant liver tumours (Magee & Barnes, 1956). It is rapidly metabolized (Dutton & Heath, 1956) and Magee (1956) demonstrated that the liver is the main, and probably the only, site of its metabolism. Magee (1957) also found that the incorporation of labelled amino acids into liver protein is impaired as early as 6 hr. after treatment.

The rapid necrotic effect on liver cells suggests that dimethylnitrosamine, or a metabolite, might interfere with respiratory metabolism of the liver cells, and this paper reports an investigation of this.

MATERIALS AND METHODS

Animals

The animals belonged to a strain of Sprague–Dawley rats inbred by brother–sister matings to the stage of successful homologous skin grafting. The diet consisted of Poultry Growers' Pellets (Barastoc Products, Melbourne), fresh green vegetables and water. Rats were used irrespective of sex when they weighed between 150 and 250 g. As far as possible, litter mates were used for each series of experiments. Dimethylnitrosamine was injected intraperitoneally in aqueous solution.

Reagents

Inorganic reagents were of analytical grade; glass-distilled water was used throughout. Cytochrome *c* was prepared by the method of Keilin & Hartree (1937) and dialysed against water. Diphosphopyridine nucleotide (DPN), adenosine 5′-phosphoric acid, adenosine 5′-triphosphoric acid (ATP), coenzyme A, L-malic acid and sodium pyruvate were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. Thiamine pyrophosphate, flavinadenine dinucleotide (FAD), thioctic acid, α-oxoglutaric acid, β-hydroxybutyric acid and choline chloride were products of L. Light and Co. Ltd. Liver concentrate 202–20 was obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. Vitamin B_{12} was a product of The Distillers Co. (Biochemicals) Ltd. Sodium citrate (A.R.), sucrose (A.R.), sodium succinate, octanoic acid, nicotinamide and the disodium salt of ethylenediaminetetraacetic acid (EDTA) were obtained from British Drug Houses Ltd. Octanoic acid was purified by redistillation *in vacuo*.

Dimethylnitrosamine was prepared by the method of Hatt (1946).

The quantity of DPN required for each experiment was weighed shortly before use and dissolved in 0·4M-nicotinamide to a concentration of 5 mg. of DPN/ml. of solution. This precaution aimed at preventing non-enzymic hydrolysis of DPN, which is otherwise rapid at neutral or acid pH, before the beginning of the incubation. A volume of 0·2 ml. of the mixture was added to each flask immediately before addition of the enzyme. In experiments in which four runs of twelve flasks had to be made in rapid succession, volumetric addition of the DPN was the most practical method.

Tissue preparations

Animals were killed by stunning and exsanguination, and the whole liver was rapidly excised and placed immediately in aqueous 0·25M-sucrose solution at 0°. After chilling, it was removed, blotted dry, weighed on a torsion balance and samples were taken for biochemical and histological studies.

Homogenates. Rat-liver homogenates (10%, w/v) were prepared in a cold room at 0–1° by means of a Potter–Elvehjem all-glass homogenizer with 0·25M-sucrose solution as the suspension medium.

Mitochondria. These were isolated by the Schneider (1948) method of differential centrifuging, in a Spinco model L refrigerated centrifuge. The centrifuge tubes and rotor were cooled before use and loaded in the cold room. The nuclei and mitochondria were washed once with, and suspended in, 0·25 M-sucrose solution. Incubations were started between 90 and 120 min. after death of the animal. Mitochondrial preparations contained between 1·4 and 1·8 mg. of N/ml. of suspension.

Analytical methods

Manometric estimations of aerobic oxidations by homogenates and mitochondrial preparations were carried out in a standard medium in which the final concentrations in the Warburg flasks were: adenosine 5-phosphoric acid, mM; KCl, 25 mM; $MgSO_4$, 6·7 mM; cytochrome *c*, 27 μM; sodium phosphate buffer, pH 7·3, 13 mM. Substrates were added to give a final concentration of 0·01 M, and 0·5 ml. of homogenate or mitochondrial suspension was used. The total volume in the flask was 2 ml. The gas phase was air and the temperature 37°. Any additions to this medium are indicated in the appropriate tables.

Succinoxidase. Activity was estimated by the method of Schneider & Potter (1943).

Choline oxidase. Activity was estimated in a medium containing: sodium phosphate buffer, pH 7·3, 0·04 M; $CaCl_2$, 0·4 mM; cytochrome *c*, 27 μM; choline chloride, 0·05 M.

Anaerobic glycolysis. Activity was estimated under the conditions used by LePage (1948).

Octanoate and pyruvate oxidations. These were 'primed' by 1 mM-malate, the oxygen uptake from which was tested in separate flasks and subtracted from the total in all results quoted.

Nitrogen. This was estimated colorimetrically after Kjeldahl digestion. The Nessler reagent was prepared according to the method of King & Wootton (1956).

Histological preparations

Samples of all livers from animals poisoned with dimethylnitrosamine, and from many of the control animals, were examined histologically. The blocks were fixed in 10% (v/v) formalin buffered to neutral pH with sodium acetate. Frozen sections cut at 7 μ by the method of Louis (1957) were stained with Sudan black, Fat red [each a saturated solution of the dye in 70% (v/v) ethanol–water] and with aqueous 1% (w/v) Nile blue sulphate. The acid haematein method for phospholipid (Baker, 1946) was also used on a special group of sections. Paraffin sections were cut at 7 μ and stained with haematoxylin and eosin, and by the Picro–Gomori, Weigert fibrin, Ziehl–Neelsen, periodic acid Schiff, Mallory phosphotungstic acid haematoxylin and methyl green pyronin methods (Lillie, 1954).

RESULTS

Histological changes

The livers of animals which had received 100 mg. of dimethylnitrosamine/kg. body weight showed consistent histological changes. By the fourth hour the cytoplasm of the cells of the inner two-thirds of the lobules was swollen and contained numerous small vacuoles of fat. Mitochondrial staining was greatly reduced. By the eighth hour the cells of the outer zone also showed these changes, and in the middle and inner zones generalized nuclear swelling and isolated necrosis of single cells or of small groups of cells were present. Confluent necrosis of the inner zone was present by the sixteenth hour, and of the middle zone by the twentieth hour. At 24 hr. only a periportal rim of swollen vacuolated cells survived. No regeneration was observed. Mitochondrial-phospholipid staining was not lost until immediately before necrosis.

In addition to fat vacuoles, spheroidal homogeneous inclusions not previously reported were consistently observed in the cytoplasm and occasionally in the nucleus. These stained magenta by the Picro–Gomori method, violet by the Weigert method, red by the periodic acid Schiff method and dark blue by the Mallory method. They did not fluoresce in blue nor in ultraviolet light, contained no stainable phospholipid, but were weakly acid-fast.

Experiments with homogenates

Homogenates were used for the initial experiments. The results shown in Table 1 were obtained at a dosage of 60 mg./kg. body weight. Consider-

Table 1. *Oxidation of various substrates by liver homogenates from control and dimethylnitrosamine-poisoned rats* (60 *mg./kg. body wt.*)

Activities are expressed as μl. of O_2/g. body wt./30 min. Each value is the average of three experiments; variation within ± 10%. Systems are described in Methods. Blank values were not subtracted.

	Control	12 hr.	16 hr.	24 hr.	30 hr.
Pyruvate	49	19	16	10	0
Octanoate	87	42	10	17	0
L-Malate	116	86	68	80	22
Citrate	94	80	53	65	11
L-Glutamate	123	82	76	77	17
α-Oxoglutarate	127	90	71	72	28
β-Hydroxybutyrate	97	63	55	61	35
Succinate	427	406	330	372	306
Choline	92	54	35	41	48
Blank	42	34	27	37	0

Table 2. *Oxidation of various substrates by liver homogenates from control and dimethylnitrosamine-poisoned rats* (100 *mg./kg. body wt.*)

Activities are expressed as μl. of O_2/g. body wt./30 min. Each value is the average of three experiments; variation within $\pm 10\%$. Systems are described in Methods. Blank values were not subtracted.

	Control	12 hr.	16 hr.	20 hr.
Pyruvate	49	12	15	6
Octanoate	88	27	2	11
L-Malate	116	78	63	41
Citrate	94	76	48	28
L-Glutamate	120	79	55	25
α-Oxoglutarate	125	105	60	30
β-Hydroxybutyrate	97	76	40	35
Succinate	424	421	395	428
Choline	92	65	90	66
Blank	42	37	25	17

able reduction of oxygen uptake with all the substrates tabulated except succinate and choline was evident by the twenty-fourth hour and was well developed by the thirtieth hour.

The 30 hr. required for well-developed biochemical changes was considered to be an undesirably long period, because of the possibility that recovery or regenerative processes, if such occur, might by then be expected to have become established. It was therefore decided to use a higher dose in order to accelerate the toxic effect and reduce the variation of response of individual animals.

Table 2 shows that when a dosage of 100 mg./kg. body weight was used, the reduction of oxygen uptake with many substrates was advanced at 20 hr. Histologically, at this time, all cells showed obvious signs of damage and some were severely affected. Individual variation was also reduced.

Oxygen uptakes with pyruvate and octanoate were inhibited at 12 hr. after administration of dimethylnitrosamine. Oxidation of the Krebs-cycle intermediates, and of L-glutamate and β-hydroxybutyrate, showed a more gradual decline although inhibition was advanced at 20 hr. Choline oxidation at 12 hr. was reduced below the control level, but there was no subsequent fall in oxygen uptake. Succinate oxidation was not reduced.

Experiments with mitochondria

The oxygen uptakes recorded in experiments with mitochondria isolated from the livers of animals killed at various intervals after poisoning with dimethylnitrosamine are shown in Table 3.

Four flasks were used for each substrate; the first pair contained the standard medium, and to the second pair was added approximately 1 mg. of DPN. The substrates were tested in successive groups of three at hourly intervals in the order shown in Table 3.

Table 3. *Effect of diphosphopyridine nucleotide on respiration of liver mitochondria from control and dimethylnitrosamine-poisoned rats* (100 *mg./kg. body wt.*)

Systems are described in Methods. The first value for each substrate is the oxygen uptake by the preparation (expressed as μl./mg. of N/hr.) without added DPN in the medium; the second value is the oxygen uptake by the preparation when 1 mg. of DPN and nicotinamide to a final concentration of 0·04 M had been added to the medium. The control values are averages of three experiments, and the values for dimethylnitrosamine-poisoned preparations are averages of two experiments at each time period. Duplicate flasks were used in each experiment.

Time after administration (hr.)	Pyruvate	Octanoate	L-Malate	Citrate	L-Glutamate	α-Oxo-glutarate	β-Hydroxy-butyrate	Choline	Succinate
Control	193 (208)	283 (265)	218 (272)	337 (403)	294 (361)	292 (323)	161 (183)	190 (274)	1300
9¼	110 (150)	179 (159)	164 (256)	178 (364)	262 (409)	273 (364)	142 (189)	173 (251)	1450
12	106 (210)	226 (253)	163 (286)	232 (427)	255 (425)	281 (368)	142 (222)	200 (318)	1640
14¼	104 (126)	0 (0)	5 (119)	11 (196)	17 (337)	35 (310)	0 (144)	137 (171)	1520
17	13 (165)	2 (10)	9 (127)	21 (236)	9 (368)	6 (283)	0 (123)	163 (225)	1640
20	0 (0)	0 (0)	0 (47)	0 (108)	0 (64)	6 (14)	0 (106)	164 (200)	1460

Only slight reductions of activity were recorded with mitochondrial preparations from animals killed up to 12 hr. after injection of dimethylnitrosamine. With all substrates except succinate and choline, considerable reductions were recorded with preparations from animals killed at 14 hr., and with preparations made at 15 hr. and subsequently, activity was negligible. This rapid loss of activity contrasted with the behaviour of the homogenates at corresponding times between dimethylnitrosamine administration and death of the animal. In general, successively smaller oxygen uptakes were recorded between 12 and 20 hr. with the homogenates, but even at 20 hr. they still retained considerable activity.

The addition of DPN to the medium increased the oxygen uptake of mitochondria from control animals. A considerable rise (44 %) was recorded with choline as substrate, and a moderate rise (10–25 %) with L-malate, citrate, L-glutamate, α-oxoglutarate and β-hydroxybutyrate. No increase was recorded with octanoate.

The oxygen uptakes of mitochondrial preparations from the livers of animals killed up to 12 hr. after poisoning by dimethylnitrosamine were raised to control values by DPN. Mitochondria from preparations made after 14 hr. which, in the absence of DPN showed negligible respiratory activity compared with that of preparations made after 12 hr., recorded oxygen uptakes only slightly lower than those of the preparations made after 12 hr. when the incubations were carried out in a medium containing DPN.

With mitochondria from animals killed at times between 15 and 20 hr. after dimethylnitrosamine poisoning, successively lower oxygen uptakes were recorded in the presence of DPN, but these were considerable when compared with the virtual absence of activity shown by the same preparations in a medium to which DPN had not been added.

Oxygen uptake with octanoate as substrate was not enhanced by DPN. Mitochondrial succinate and choline oxidation, in agreement with the results of the homogenate experiments, remained at normal levels throughout the 20 hr. period. The choline oxidase activity of normal mitochondria was enhanced when DPN was added to the incubation medium. This was also observed with mitochondria from rats poisoned with dimethylnitrosamine over the 20 hr. period, and the increase was similar in degree to that shown by the control preparations.

Effects of other cofactors

Experiments were carried out with liver mitochondria from animals injected with dimethylnitrosamine 14–15 hr. before killing, to determine whether an increased oxygen uptake could be obtained with cofactors other than DPN. Pyruvate, octanoate, and α-oxoglutarate were used as substrates, and two cofactor mixtures were made. With the first mixture the addition to each flask contained coenzyme A 0·1 mg., thiamine pyrophosphate 0·2 mg., thioctic acid 0·1 mg., ATP 1 mg., and vitamin B_{12} 5 μg. With the second mixture the addition to each flask contained ATP 1 mg., FAD 20 μg. and crude liver concentrate 2 mg.

No increase of oxygen uptake with any substrate was obtained with either mixture. In order to ensure that this result was not due to unsatisfactory mitochondrial preparations, additional flasks containing α-oxoglutarate as substrate and 1 mg. of DPN in the standard medium were also incubated. In each experiment the mitochondrial preparation was capable of a considerably increased oxygen uptake when DPN was present.

Thus, of the various cofactors tested, only DPN produced any effect.

Anaerobic glycolysis

Experiments were carried out to determine whether dimethylnitrosamine poisoning reduced the capacity of the liver to carry out anaerobic glycolysis in addition to aerobic oxidation (Table 4).

No impairment was demonstrable even at 22 hr., although at this time the activity of many of the enzymes of the tricarboxylic acid cycle had fallen to negligible levels (Table 2).

Effect of dimethylnitrosamine in vitro

Experiments were carried out to determine whether dimethylnitrosamine was capable of exerting an effect *in vitro* when added to an homogenate of normal rat liver (Table 5).

No significant reduction of activity of the preparation treated with dimethylnitrosamine occurred.

Table 4. *Anaerobic glycolysis by liver homogenates from control and dimethylnitrosamine-poisoned rats* (100 *mg./kg. body wt.*)

The medium was that of LePage (1948), and the homogenate addition was 0·3 ml. in a final flask volume of 2 ml. During the 5 min. equilibration period, a continuous stream of $N_2 + CO_2$ (95:5) was passed through the flasks. Incubation was carried out at 37° and readings were taken during the first 15 min. Activities are stated as CO_2 production (μl.) by liver tissue/g. body wt./15 min. Each figure represents the average of three experiments.

Time after administration (hr.)	Activity
Control	84
11	89
17	81
22	77

Table 5. *Effect of dimethylnitrosamine* in vitro

In each experiment at 10% (w/v) homogenate in 0·25 M-sucrose solution was prepared from the liver of a normal rat and divided into two portions: dimethylnitrosamine was mixed into one portion to yield a final concentration of 0·15 M. The treated and untreated portions of the homogenate were stored at 0° for the stated periods before the determination of oxygen uptakes, or centrifuging for the preparation of mitochondria. Oxygen consumption was determined in the manner described in Methods. Readings were taken over a 30 min. period.

Expt. no.	Time of exposure of treated sample to dimethylnitrosamine (hr.)	Preparation	Substrate	Oxygen uptake (μl./30 min./50 mg. wet wt. of liver): Untreated	Dimethylnitrosamine-treated
1	1	Homogenate	Octanoate	150	146
	7	Homogenate	Octanoate	135	132
	1	Homogenate	α-Oxoglutarate	117	113
	7	Homogenate	α-Oxoglutarate	104	102
2	1	Homogenate	Pyruvate	116	113
	5	Homogenate	Pyruvate	104	99
	1	Homogenate	Citrate	84	79
	5	Homogenate	Citrate	78	73
3	3	Homogenate	Octanoate	150	146
	3	Homogenate	L-Glutamate	110	107
	4	Mitochondria	Octanoate	102	102
	4	Mitochondria	L-Glutamate	69	71

Table 6. *Effect of ethylenediaminetetra-acetic acid on oxygen uptakes of liver mitochondria from control and dimethylnitrosamine-poisoned rats* (100 *mg./kg. body wt.*)

Two homogenates (10%, w/v) were made from each liver, one in 0·25 M-sucrose solution and the other in 0·25 M-sucrose solution containing 6 mM-EDTA. Mitochondria were prepared from these homogenates and were suspended in 0·25 M sucrose solution. Oxygen uptakes were measured as described in Methods over a period of 30 min.

		Oxygen uptake (μl./g. body wt./30 min.)			
		Mitochondria from control animals		Mitochondria from animals killed 16 hr. after dimethylnitrosamine injection	
Expt. no.	Substrate	Sucrose preparation	EDTA–sucrose preparation	Sucrose preparation	EDTA–sucrose preparation
1	Pyruvate	24	—	0	10
2		37	—	29	21
3		56	60	1	3
2	Octanoate	51	—	45	43
3		92	93	1	1
4	L-Malate	28	35	4	5
1	Citrate	29	—	2	11
2		43	—	26	31
3		75	80	2	13
4	L-Glutamate	28	43	3	7
1	α-Oxoglutarate	34	—	2	11
2		41	—	35	37
3		62	71	3	28
4		36	44	4	9
4	β-Hydroxybutyrate	29	36	2	2

Effect of ethylenediaminetetra-acetic acid

Gallagher, Gupta, Judah & Rees (1956) demonstrated that the presence of EDTA in the medium reversed inhibitions of citrate, α-oxoglutarate and pyruvate oxidation by liver preparations from thioacetamide-poisoned rats.

Table 6 indicates that no effect comparable with that shown for thioacetamide poisoning occurred with dimethylnitrosamine.

DISCUSSION

The selective, acute toxic action of dimethylnitrosamine on the liver, in which an advanced lesion can be produced before the animal dies, makes it a useful agent in the investigation of the mechanism of cellular injury. The liver is also a convenient tissue, having been extensively investigated in the normal state.

In a similar study of the action of the pyrrolizidine alkaloid heliotrine, Christie (1958) discussed the desirability of adjusting the dose to obtain a lesion in which all the cells of the organ were affected by the pathological process to an equal extent. Dimethylnitrosamine has a zonal effect at low dosage but selection of an appropriate higher dose (100 mg. of dimethylnitrosamine/kg. body weight) produced a reasonable approximation to a state of homogeneity, thus avoiding admixture of the damaged cells with normal, completely necrotic or regenerating cells. Histological examination of livers on which biochemical determinations were made was valuable in selecting the optimum dose and in determining the range of individual variation in the response of the animals to that dose.

A standard which permits comparison of data from different animals is essential in studying the evolution of a toxic process over a period of time. In work on heliotrine (Christie, 1958) it was found advantageous to refer the assay data to the wet weight (g.) of liver tissue present/g. body weight of the animal. This standard again proved useful for comparing homogenate activities. In the mitochondrial experiments it was found that activities could be satisfactorily referred either to the body-weight standard or to mitochondrial nitrogen. The more commonly used standard was therefore chosen in constructing Table 3.

The present results showed that a condition of virtual DPN deficiency of several mitochondrial DPN-linked respiratory enzymes occurred during the course of dimethylnitrosamine poisoning. There was an almost simultaneous loss of activity of these enzymes beginning after a latent period of some 12 hr. after administration of the toxic dose, and progressing to an advanced stage by 20 hr. Reactivation could, for a time, be obtained by the addition of DPN to the medium. No similar effect could be obtained with any of the other cofactors tested.

Some of the DPN-linked systems affected were steps of the Krebs cycle, and thus oxidative metabolism became disorganized over the period between 12 and 20 hr. It will be recalled that a rapid advance of the histological lesion from one of generalized swelling and vacuolation of the cells to extensive necrosis occurred during this period. However, histological signs of cell injury were widespread before 12 hr. It therefore seems reasonable to suggest that the terminal stage of mitochondrial DPN deficiency is preceded by earlier biochemical processes which culminate in this condition.

The DPN-deficiency effect could be due to leakage of DPN from the mitochondria comparable with the 'ageing' effect with normal mitochondria (Ernster, 1956) and with the state induced by carbon tetrachloride poisoning (Christie & Judah, 1954). Alternatively, DPN might be inactivated *in situ* at the mitochondrial enzymic foci, or in transit to these sites. Finally, there might be an impairment of the synthesis of DPN, which is largely a nuclear activity (Hogeboom & Schneider, 1952; Preiss & Handler, 1958). These possibilities are under investigation.

Survival of the glycolytic and succinoxidase systems was also observed in heliotrine poisoning (Christie, 1958). With both toxic agents, the level of activity of the glycolytic and succinoxidase systems was essentially unchanged long after most of the liver cells were histologically dead. Enhanced activity of surviving cells seems an unlikely explanation for the maintenance of a steady level throughout the course of gross disorganization by two dissimilar agents. On the other hand, both systems are notable for a resistance to disruptive fractionation methods which is not usually found with systems of equivalent complexity. It was therefore concluded, both with dimethylnitrosamine and with heliotrine, that cell death did not alter succinoxidase and glycolytic capacity of the liver over the duration of the experiments.

Some toxic agents which have been investigated along the present lines have shown the capacity to exert an action on normal liver preparations *in vitro*. Dimethylnitrosamine did not show a direct effect on homogenates of normal rat liver *in vitro*. This supports the evidence of Magee (1956) that the actual hepatotoxic agent is not dimethylnitrosamine itself but a metabolite which is formed from it in the liver.

The failure of EDTA to reactivate homogenate or mitochondrial preparations excluded the possibility that the suggested mechanism of action of

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 cofactor 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

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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.

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