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ENZYMES ACTING ON GLUCOSAMINE PHOSPHATES *

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Uridine disphosphate acetylglucosamine was first isolated from yeast [1] and has been detected in mammalian liver [2 3 4 5] and hen oviduct [6] A specific pyrophosphorylase has been found [5] to transform it into uridine triphosphate and acetylglucosamine-1-phosphate, so that the latter is likely to be a normal metabolite in mammals. In order to make this ester available for enzymic studies, a chemical method for its synthesis has been developed, following a procedure inspired by that used by CORI, COROWICK AND CORI [7] for the preparation of glucose-1-phosphate.

Acetylglucosamine-1-phosphate was first tested with an enzyme from *Neurospora* and found [8] to be converted to acetylglucosamine-6-phosphate. This enzyme which is activated by the 1,6-disphosphates of glucose or acetylglucosamine has been studied by REISSIG [9]. It has now been observed that similar changes are catalyzed by mammalian enzymes thus giving rise to acetylglucosamine-6-phosphate. This substance which has been previousty obtained by the enzymic acetylation of glucosamine 6-phosphate [8 10] has been found to be converted into fructose-6-phosphate by kidney enzymes. But the most unexpected finding was that it catalyses the enzymes transformation of glucosamine-6-phosphate into fructose-6-phosphate and ammonia. Furthermore the latter reaction was found to be reversible so that it affords a possible route to hexosamine synthesis different from that found in *Neurospora* [8] where glutamine is involved. Previous work on hexosamine metabolism has been reviewed by DORFMAN [11] and KENT AND WHITEHOUSE [12].

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METHODS

Analytical

The following methods were used. Phosphate. FISKE AND SUBRAROW [13], protein, KUNITZ AND MCDONALD [14]; fructose, ROE [15]; glucosamine, BLIX [16]; *ammonia*, CONWAY [17]; for acetylglucosamine a modification of the MORGAN AND ELSON method [18] was used with a standard of acetylglucosamine prepared as described by ROSEMAN AND LUDOWIEG [19]. With purified enzymes deproteinization was unnecessary. For acetylglucosamine-1-phosphate which does not give the test directly-proteins were precipitated with 5 % trichloracetic acid. After centrifugation, 0.5 ml. of supernatant was heated 10 minutes at 100°C in orden to hydrolyze the phosphate group. After cooling, 0.15 ml of 1.*M* *potassium* borate was added and the procedure continued as described by REISSIG *et al* [18] *A* larger amount of borate (0.15 instead of 0.1 ml) was used in order to neutralize the acid.

When it was necesary to distinguish free hexosamines from their phosphoric esters (Table III) the latter were precipitated by adding zinc sulfate and barium hydroxide solutions as described by SOMOGYI [20].

Estimation of the enzymes

The test system for measuring the disappearance of acetylglucosamine-6-phosphate contained 0.1 μmole of substance 0.02 ml of 1*M* tris-hydroxymethylaminomethame buffer of pH 7.7 and the enzyme.

For glucosamine -6-phosphate the system contained 0.1 μmole of *N* glucosamine-6-phosphate, 0.02 ml of 1 *M* tris buffer of pH 8 4, 0.02 μmole of acetylglucosamine-6-phosphate and the enzyme.

For both tests the total volume was 0.05 ml and the incubation time: 15 minutes at 37°C *A* unit was defined as the amount of enzyme causing the disappearance of 25 % of the substrate in 15 minutes.

Preparation of acetylglucosamine-I-phosphate

This substance was prepared by making trisilver phosphate react with chlorotetraacetylglucasamine. An amorphous bromo derivative was used in many cases until Dr. R. JEANLOZ suggested the use of the chloro compound which can be obtained crystaline.

a-I-chloropentacetylglucosamine. The procedure described by BAKER *et al.*[21] was used with minor modifications. Pentacetylglucosamine (6.2 g) prepared with zinc chloride as catalyst as described by LEVENE [22] was added to a solution obtained by mixing 104 ml of dry ethyl ether saturated with HCl at 0°C. 18.7 ml

of acetic acid anhydride and 6 ml of glacial acetic acid. After keeping for 2-3 days at 0°C with occasional shaking, the solid was dissolved. The solution was concentrated *in vacuo* below 15°C. The solid was then dissolved in chloroform and treated as described by BAKER *et al.*[21].

Phosphorylation. 2.9 g of chloropentaacetylglucosamine were mixed with 1.2 g of trisilver phosphate[23] and 60 ml of dry benzene. This mixture was heated in a water bath and about 5 ml of the benzene was distilled in order to remove traces of water. After this the heating was continued during 15 minutes, under reflux with shaking. The solid changed from yellow to white and the liquid became brownish.

The mixture was filtered hot through filter aid (celite) and washed with hot benzene.

Hydrolysis. The benzene solution was concentrated to dryness *in vacuo* and the solid was dissolved in 30 ml of methanol containing 1.2 ml of 5 *N* sulfuric acid. After keeping it for 30 minutes at 37° C the pH was adjusted to about 8.9 (thymol blue) with 10 *N* sodium hydroxide. The pH was kept alkaline during a few hours by occasional addition of sodium hydroxide. An excess of 50 % barium acetate was added followed by 30 ml of ethyl ether. After leaving it overnight at 5°C the mixture was centrifuged. The precipitate was extracted several times with water. The pooled water extracts contained 730 μmoles of labile phosphate.

Purification. A solution containing about 100 μmoles of labile phosphate adjusted to pH 8 was poured onto a column (90 cm × 1.8 cm^2) of Dowex 1 of 10 % crosslinkage in the chloride form. Gradient elution was carried out as described by ALM *et al.* [24]. A solution of 0.005 *M* HCl in 0.1 *M* $CaCl_2$ was allowed to enter into a 250 ml mixing chamber filled wtih water. The fractions (5 ml) were analysed for acetylglucosamine, after heating for 10 minutes at 100°C in 0.1 *N* acid, and for labile phosphate. Several well separated peaks appeared. The first was free acetylglucosamine. The second had a ratio acetylglucosamine/labile phosphate of 1.7 to 1.9 and consisted mainly of a diester. The third peak which was the largest contained acetylglucosamine-1-phosphate. Finally a small peak containing unidentified substances appeared.

The fractions corresponding to the third peak were pooled, neutralized with solid $Ca(OH)_2$, filtered, concentrated *in vacuo* to 1-2 ml and precipitated with 3 volumes of ethanol. If necessary the precipitation was completed by the addition of ethyl ether. The solid was washed several times with ethanol in order to remove the calcium chloride, washed with ethyl ether and dried. Usually the solid was dissolved in a small amount of water, centrifuged and precipitated with ethanol. The yield was about 50 % of the labile phosphate introduced into the column. The ratio acetylglucosamine/phosphate was about 1 and the purity with respect to dry weight was 84 %.

The rotatory power was measured on 0.3 ml of solution containing 39 μmoles of he calcium salt and the concentration was checked by phosphate estimation. $[\alpha]_D = +107$.

Preparation of glucosamine-6-phosphate

Glucosamine was phosphorylated with ATP by a procedure similar to that described by BROWN [25]. The following mixture was incubated at pH 8: 220 μmoles of glucosamine hydrochloride, 120 μmoles of ATP, sodium salt, 1 ml of 0.1 *M* magnesium sulfate and 1 ml of Lebedew juice (prepared by extracting dry yeast with 3 volumes of 0.1 *M* sodium bicarbonate during 24 hours at 5° C) and water to complete 10 ml. After 1 hour at 35° C the proteins were coagulated by heating and centrifuged off .

The filtrate was then poured into a column 1.8 cm^2 X 100 cm of Dowex-1 (X 10) in the acetate form. Elution was carried out by a gradient obtained by allowing 0.05 *N* acetic acid to flow into a mixing chamber containing 250 ml of water. The fractions were analysed by the method of BLIX [16].

The first fractions contained glucosamine while the phosphate ester appeared later and was well separated from the former. The last fractions of glucosamine phosphate were slightly contaminated with a substance absorbing at 200 mμ and were rejected.

The pooled fractions of glucosamine phosphate were concentrated *in vacuo* to about 3 ml, the pH was adjusted to 7.2 with barium hydroxide and 3 volumes of ethanol were added. The precipitate was separated, washed with ethanol and ether and dried. The yield was 60-70 % of the glucosamine used, and the product was 80 % pure.

Preparation of N-acetylglucosamine-6-phosphate

Glucosamine-6-phosphate was treated with acetic anhydride as described by ROSEMAN [26]. The whole reaction mixture including the resin used for the acetylation was poured on top of a column of Dowex-1 chloride. Displacement from the column was effected gradient-wise as described for acetylglucosamine-1-phosphate, but using 0.15 *N* HCl. The fractions which gave a ratio total phosphate/acetylglucosamine of one were pooled and the calcium salt was obtained as described for the 1-phosphate. The yield was rather low owing to losses occurring in the precipitation with ethanol.

In some experiments acetylation was carried out at 0°C with a slight excess of acetic anhydride in aqueous-pyridine solution. The results were essentially the same as with the other procedure.

N-propionylglucosamine-6-phosphate

The procedure was the same as for the acetyl derivative but propionic acid anhydride was used.

Purification of the enzyme acting on the 6-phosphates

Pig kidneys obtained frozen from the slaughterhouse yielded active extracts even after several weeks storage in the frozen state. The cortex was homogenized with a blendor in 3 volumes of water and the mixture was centrifuged at 3000 r.p.m. in the cold for 10 minutes (crude extract).

To 80 ml of the crude extract 40 ml of ammonium sulfate solution (50 g percent w/v) were added. After 10 to 15 minutes at 5°C the precipitate was centrifuged off and discarded. To 100 ml of the supernatant 20 ml of ammonium sulfate solution were added. The precipitate was separated, dissolved in water and dialysed 4-5 hours at 5°C (Fraction A). Ammonium sulfate solution (0.2 vol) was added to the supernatant of the previous step. The precipitate was redissolved and dialysed (Fraction B).

Fraction B was treated with a solution of yeast nucleic acid adjusted to pH 7 (100 mg per g of protein). By cautious addition of dilute HCl followed by contribution, several fractions were obtained (B_1 B_2, *etc.*). The precipitates were suspended in water and neutralized. The results are shown in Table I.

TABLE I

Purification of the enzymes

Fraction	Volume ml	Protein mg/ml	Glucosamine-6-P		Acetylglucosamine-6-P		Ratio A / B
			Units/mg A	Total units	Units/mg B	Total units	
Crude extract	80	53	10	43000	0.75	3200	13.3
Fraction A	5	60	30	9000			
Fraction B	3.5	50	40	7000	3.8	650	10.5
Fraction B_1	0.23	50	30	350			
Fraction B_2	0.35	70	75	1800			
Fraction B_3	0.30	58	196	3500	5.0	88	39
Fraction B_4	0.30	46	93	1300			

RESULTS

Prosperties of acetylglucosamine-I-phosphate

The molecular rotation of I-phospho sugars is known to be comparable to that of the methylglycosides (*cf.* LELOIR [27]). For methyl-N-acetylglucosaminide the values given by Neuberger and Pitt Rivers [28] are + 24,675 for the α- and 10.105 for the β-anomer. The acetylglucosamine-1-phosphate prepared as described here gave a value of + 36,000 so that it is the α-anomer. The product has been used by Dr. REISSIG [9] in a study of the phosphoacetylglucosamine mutase of *Neurospora* and several preparations were found to be over 90 % converred into the 6 phosphate. The substances does not reduce sugar reagents and gives the Morgan and Elson reaction only after acid hydrolysis.

TABLE II

Acid hydrolysis of acetylglucosamine-1-phosphate

Time (hours)	Acetylglucosamine-1-phosphate		Glucose-1-phosphate	
	Percent hydrolysis	10^3K	Percent hydrolysis	10^3K
1	26.6	2.2	37.8	3.3
2	41.0	1.9	60.5	3.4
3	58.2	1.7	76.2	3.4
4	58.2	1.6	79.0	2.8
5	67.8	1.7	87.0	2.9
6	75.4	1.7	92.3	3.1
18	100.0	—	100.0	—
	Mean	1.8		3.1

Inorganic phosphate was estimated after incubating the samples at 37°C in 1 *N* sulfuric acid. The formula used was: $K = (t_2 - t_1)^{-1} \log_{10} (100 - x_1) / (100 - x_2)$. The time was taken in minutes. No detectable amounts of glucosamine were formed.

The results of acid hydrolysis in 1 *N* acid at 37°C are shown in Table II. It may be observed that the acetylglucosamine ester is slightly more stable than that of glucose. However it is much more labile than glucosamine-1-phosphate [29] and galactosamine -1-phosphate [30]

The action of enzymes on acetylglucosamine-1-phosphate

On incubation of the 1-phospho ester with crude kidney or liver extracts it was observed that a part was converted to free acetylglucosamine while another part was transformed into substances which did not give the Morgan and Elson test even after acid hydrolysis. The formation of acetylglucosamine-6-phosphate as an intermediate in this reaction could be detected only by purification of the crude extracts or as shown in Table III by the use of an inhibitor. Such a selective inhibition of acetylglucosamine-6-phosphate-disappearance could be obtained with acetate without affecting the activity on the 1-phosphate. Under these conditions about half of the decrease in acetylglucosamine-1-phosphate could be accounted for by the increase in the 6-phosphate. These results are shown in Table III. Other test were carried out with an enzyme prepared by a method based on the first steps of the purification of phosphoglucomutase as described by NAJJAR [31]. The results in Table IV show that the process is activited by magnesium ions and slightly activated by glucose disphosphate. In that experiment the results of the estimation were checked with a specific enzyme method for acetylglucosamine-6-phosphate. In the other experiments rat muscle or liver extracts were found to catalyse the conversion of the 1-to 6-phosphate at similar rates.

The action of enzymes en the 6-phosphates

Crude preparations of pig kidney catalyse the disappearance of acetylglucosamine-6-phosphate. Glucosamine-6-phosphate also disappears provided small amounts of acetylglucosamine-6-phosphate are also added. As shown in Table I both activities may be detected after some purification but the ratio of the two varies from 13 to 39. The action on the non-acetylated substance was always higher than on the acetylated.

TABLE III

The action of kidney extracts on acetylglucosamine-1-phosphate

	Δ in micromoles	
	No acetate	*With acetate*
Acetylglucosamine-1-phosphate (c—a)	— 0.053	— 0.063
Free acetylglucosamine (b)	+ 0.015	+ 0.017
Acetylglucosamine-6-phosphate (a—b)	+ 0.002	+ 0.033

Incubation of 0.05 ml of crude kidney extracts (1 vol of water used) 0.01 ml of 0.36 *M* glycerophosphate buffer pH 7.4, 0.01 ml of 1 *M* sodium acetate (pH 7.4) and 0.08 μmole of acetylglucosamine-1-phosphate Estimations were as follows: (a) direct estimation of acetylhexosamine, (b) same in the supernatant obtained after precipitation with zinc sulfate and barium hydroxide, (c) as (a) but after removing protein with 5 % trichloracetic acid and heating 10 minutes at 100°C.

TABLE IV

The conversion of acetylglucosamine-1-phosphate to the 6-phosphate

	μ moles of acetylglucosamine-6-phosphate	
	Chemical method	*Enzymic method*
Complete system	0.083	0.08
No glucose diphosphate	0.076	—
No magnesium	0.045	—

The complete system contained 0.01 ml of 0.36 *M* glycerophosphate buffer pH 7.4, 0.005 ml of saturated solution of 8'-hydroxyquinoline, 0.01 ml. of 0.1 *M* magnesium chloride, 0.005 μmole of glucose diphosphate, 0.17 μmole of acetylglucosamine-1-phosphate and enzyme. Incubated 1 hour at 37°C, total volume 0.08 ml. The enzyme was prepared from pig kidney by adjusting the crude extract to pH 5, precipitating the supernatant with 0.65 saturated ammonium sulfate and dialysing. Estimations by chemical method as in Table III. For the enzymic method the activating power on glucosamine-6-phosphate disappearance was compared with a standard of acetylglucosamine-6-phosphate using a purified enzyme.

The pH optima are shown in Fig. 1.

Free glucosamine or acetylglucosamine were not acted upon by the enzyme preparations.

Many experiments we carried out in order to detect a cofactor. The enzyme was submitted to prolonged dialysis against ethylenediaminetetracetate solutions, to precipation with 0.1 *N* acid from ammonium sulfate solutions and to treatment with anion exchange resins but no organic or inorganic ion requiriment could be detected.

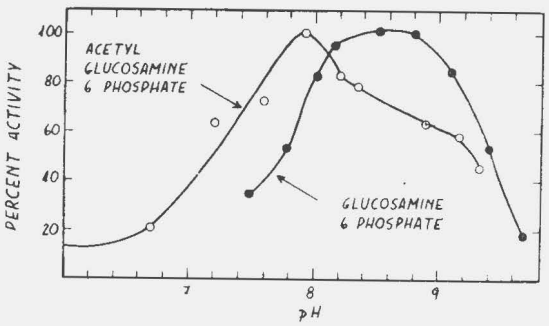


FIG. 1. — pH optima. Conditions as described for test system with Tris HCl and Tris-maleate, buffers prepared as described by GOMORI 35. The pH was checked in aliquots with a glass electrode.

The action of acetate

As shown in Table V acetate nearly suppresses acetylglucosamine-6-phosphate disappearance while it does not affect appreciably that of glucosamine-6-phosphate. The results with propianate or butyrate were similar to those obtained with acetate while formate showed no action.

TABLE V

The action of acetate

Substrate	*Addition*	*Substrate* (γ *mole*)
Acetylglucosamine-6-phosphate	none	0.09
Acetylglucosamine-6-phosphate	acetate	0.02
Glucosamine-6-phosphate	none	0.14
Glucosamine-6-phosphate	acetate	0.14

Conditions as described for test system with 0.2 *M* acetate.

The action of acetylglucomasine-6-phosphate on glucomasine phosphate disappearance

As shown in Fig. 2 glucosamine-6-phosphate is not transformed in the absence of acetyl-

glucomasine-6-phosphate. The effect of the latter is catalytic since 0.009 μmole can produce the disappearance of 0.05 μmole of glucosamine-6-phosphate. Many substances have been tested as possible substitutes for acetylglucosamine-6-phosphate. The results were negative with: acetylglucosamine, acetamide, UDP-acetylglucosamine, acetate, acetate plus ammonia, hexose-6-phosphates, acetylglycine, acetyltriptophane, acetylcholine, pyruvate, citrate and α-Ketoglutarate. With acetylglucosamine-1-phosphate some activation was obtained using crude extracts but none with purified preparations.

Only one substance was found to have the activity of N-acetylglucosamine-6-phosphate and that was N-propionylglucosamine-6- phosphate. This ester was also found to disappear on incubation with the ensyme. The quantitative results were very similar with the acetyl and propionyl derivatives.

te the reaction was faster so that the interference of traces of isomerase was smaller. Fig. 3 shows the result of such an experiment. Glucosamine-6-phosphate disappeared rapidly with a concomitant rise in the fructose values and in the sum fructose-6 plus glucose-6-phosphate as deteminated whit glucose-6-phosphate dehydrogenase plus isomerase and TPN. After some minutes the fructose values decreased slowly, as was expected, owing to the presence of isomerase in the enzyme preparation.

In another experiment with a preparation that was nearly free from isomerase the results were as follows:

	Δ
Glucosamine-6-phosphate	— 0.046
"fructose"	+ 0.045
Ammonia	+ 0.048

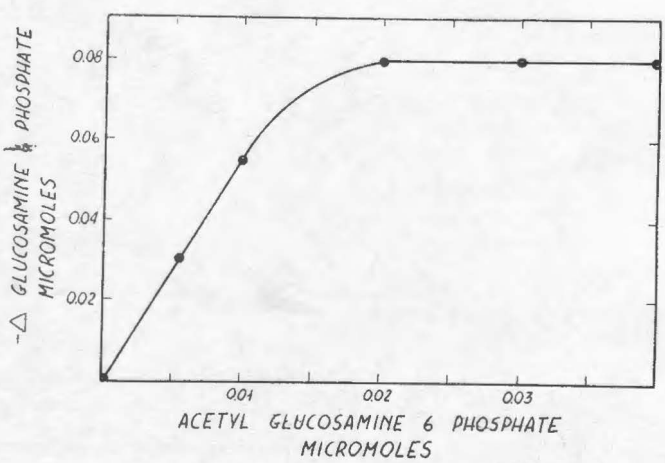


FIG. 2. — The action of acetylglucosamine-6-phosphate on glucosamine-6-phosphate disappearance. Conditions as described for test system, but with variable amounts of acetyl glucosamine-6-phosphate.

The reaction products

Analysis by paper chromatography of the products obtained from acetylglucosamine-6-phosphate with a crude enzyme preparation, and after treatment with phosphatase, revealed the presence of glucose, fructose and in some cases of a substance reacting like heptulose [32]. Hexose phosphate isomerase was dificult to remove from the preparations but in one case a quantitative accumulation of fructose ester from acetylglucosamine-6-phosphate was obtained. With glucosamine-6-phosphate as substrate

It seems clear therefore that the primary reaction products are fructose-6-phosphate and ammonia.

Reversible formation of glucosamine-6-phosphate

The incubation of fructose-6-phosphate and ammonia with the enzyme and small amounts of acetylglucosamine-6-phosphate led to a definite increase in "glucosamine" as estimated by the BLIX method. No increase ocurred

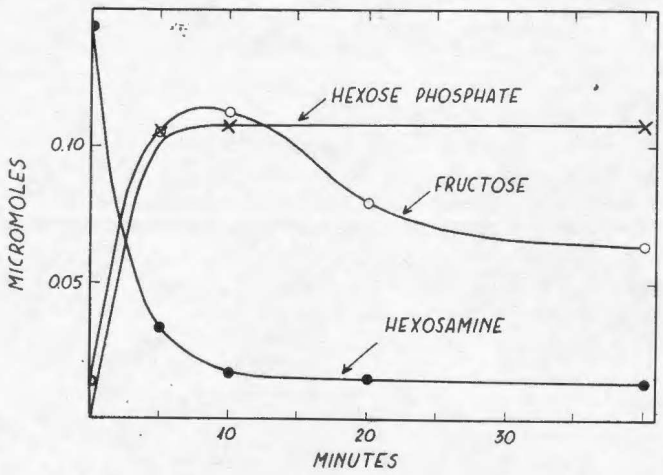


FIG. 3. — Chemical changes during the transformation of gldcosamine-6-phosphate. The reaction mixture contanied 0.15 μmole of glucosamine-6 phosphate, 0.04 μmole of acetylglucosamine-6-phosphate, 0.04 ml of 1 *M* tris buffer of pH 8.4 and 0.04 ml of enzyme precipitated twice with nucleic acid. Total volume 0.1 ml. Temperature: 37°C.

if the ammonia, fructose phosphate or acetylglucosamine-6-phosphate were omitted or if ammonia was substituted by glutamine or asparagine. The results were also negative if free fructose, glucose, xylose or ribose were added instead of fructose-6-phosphate. A representative experiment is shown in Table VI. These results were taken as an indication that the reaction can be reversed and measurements of the equilibrium constant were carried out, starting with known reaction mixtures and estimating the changes in glucosamine phosphate produced by the enzyme. One suchs experiment is shown in Table VII. The values obtained for *K* in moles/liter varied from 0.12 to o.18. In other experiments the results were more variable (0.04 to 0.19). Owing to analytical errors and to some uncertainly regarding the true molarity of the substrates perhaps the results should only be taken as indicating the order of magnitude of *K*. Thus the fructose-phosphate concentration was calculated assuming that isomerase converted it into the equilibrum mixture which contains 66 % of glucose-6-phosphate. However, it was clear in all the experiments that similar values for *K* are obtained in the forward and in the reverse reaction.

TABLE VI

Synthesis of glucosamine-6-phosphate

	"Glucosamine" formed μ *moles*
Complete system	0.014
No ammonium sulfate	0.002
No acetylglucosamine-6-phosphate	0.002
Glutamine instead of ammonium sulfate	0.002
Asparagine instead of ammonium sulfate	0.002

The complete system contained: 0.5 μmole of fructose-6-phosphate 1.5 μmoles of ammonium sulfate, 0.02 μmole of acetylglucosamine-6-phosphate, 0 01 ml of 2 *M* tris buffer and 0.27 mg of enzyme preparation (B_1 Table I). Total volume 0.05 ml, 30 minutes at 37°C. The glucosamine values obtained on samples at t_0 were substrated.

TABLE VII

Equilibrium constant

Substances added (μ *moles*)			Δ	*K*
Fructose-6-P	*Ammonia*	*Glucosamine-6-P*	*Glucosamine*	
0.326	1.0	0	+ 0.0128	0.16
0.326	1.0	0.0085	+ 0.0058	0.15
0.326	1.0	0.0170	+ 0.0016	0.12
0.163	2.0	0	+ 0.011	0.18
0.163	2.0	0.0085	+ 0.0057	0.15
0.163	2.0	0.0170	− 0.002	0.16

Incubation of fructose-6-phosphate with ammonium sulfate and glucosamine-6-phosphate; 0.02 ml-tris buffer 1 *M* pH 8.4; 0.02 mg enzyme (B_3, Table I) final volume 0.05. ml., 10 min at 37°C, $K = [NH_2] \cdot [\text{Fructose-6-phosphate}] / [\text{glucosamine-6-phosphate}]$ in moles/liter. The concentration of fructose-6-phosphate was taken as 1/3 of theorical amount in order to correct for the presence of isomerase.

TABLE VIII

The action of extracts from different organs on glucosamine-6-phosphate

Organ	*Activity units/mg protein*
Kidney	10.0
Brain	2.2
Intestine	1.0
Liver	0.5
Lung	0.4
Heart	0
Brewars yeast	0

The rat organs were homogenized in 2 vol. of water at 0°C and centrifuged. Test with glucosamine-6-phosphate were carried out as described for the test system.

Distribution of the glucosamine-6-phosphate enzyme (s)

As shown in Table VIII, kidney is the richest source of enzyme, followed by brain, intestine, liver and lung. No activity was detected in heart or in yeast. The same extracts were tested for the rate of the reverse reaction starting with fructose phosphate and ammonia and essentially similar results were obtained.

DISCUSSION

The first step in the transformation of acetylglucosamine-I-phosphate catalysed by animal tissues has been found to be:

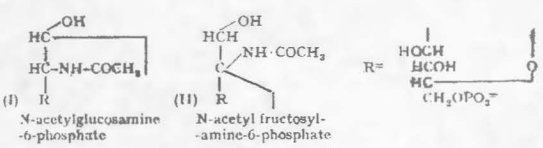
acetylglucosamine-1-phosphate $\rightleftharpoons$ acetylglucosamine-6-phosphate

It remains to be decided whether the change is brought about by phosphogluco-mutase or by a specific enzyme. Evidence showing that two enzymes are present in *Neurospora* extracts has been obtained by REISSIG [9] who separated some fractions which were more active on the acetylglucosamine ester than on glucose phosphate, and other fractions which behaved inversely. He also detected phosphoacetylglucosaminemutase activity in a highly purified rabbit muscle phosphoglucomutase.

As to the transformations of the 6-esters the reported facts can be rationalized by the following reactions:

Other formulations involving a cofactor can be written but no supporting evidence was found despite many efforts. Reactions (I) + (3) would be responsible for the disappearance of acetylglucosamine-6-phosphate. Acetate would inhibit reaction (3) -Glucosamine -6-phosphate would be transformed through reactions (I) + (2) and thus the necessity of catalytic amounts of acetylglucosamine-6-phosphate would be explained.

As to the nature of the substance X it is attractive to suppose that it is N-acetylfructosylamine-phosphate (II). In this case reaction (1) would be similar to an Amadori rearrangement, *i.e.* the conversion of an aldose (I) to a ketose (II) derivative. After transacetylation (Reaction (2)) the product would be fructosylamine phosphate, which is presumably an unstable substance that descomposes into fructose phosphate and ammonia. It may be mentioned that fructosylamine does not appear to be stable since it has never been prepared and when fructose reacts with ammonia it is the isomer, glucosamine, which has been obtained (HEYNS AND MEINECKE [33], CARSON [34]).



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SUMMARY

The chemical synthesis of α-acetylglucosamine-1-phosphate and some of its properties are described. From kidney or liver, enzymes have been obtained which

$$\text{acetylglucosamine-6-1'} \rightleftharpoons X \quad (1)$$

$$\text{glucosamine-6-P} + X \rightleftharpoons \text{acetylglucosamine-6-P} + \text{fructose-6 P} + NH_3 \quad (2)$$

$$X \rightarrow \text{fructose-6-P} + NH_3 + \text{acetate} \quad (3)$$

catalyse the following reactions:

$$\alpha\text{-acetylglucosamine-1-phosphate} \rightleftharpoons \text{acetylglucosamine-6-phosphate} \quad (1)$$

$$\text{N-acetylglucosamine-6-phosphate} \rightleftharpoons \text{fructose-6phosphate} + NH_3 + \text{acetate} \quad (2)$$

$$\text{glucosamine-6-phosphate} \rightleftharpoons \text{fructose-6-phosphate} + NH_3 \quad (3)$$

Reaction (1) was found to be activated by magnesium ions. The enzyme(s) responsible for reactions (2) and (3) were purified and it was observed that (3) requires catalytic amounts of N-acetylglucosamine-6-phosphate (or of the N-propionyl derivative) and that it is reversible. The possible mechanism of the reactions is discussed.

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