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The Tissue Content and Turnover Rates of Intermediates in the Biosynthesis of Glycosaminoglycans in Young Rat Skin

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1. The tissue contents of hexose monophosphate, *N*-acetylglucosamine 6-phosphate, UDP-glucose, UDP-galactose, UDP-*N*-acetylglucosamine, UDP-*N*-acetylgalactosamine and UDP-glucuronic acid were determined in the skin of young rats less than 1 day *post partum*. Tissue-space determinations were used to calculate their average cellular concentrations. 2. The incorporation of [U-^{14}C]-glucose into the intermediates was recorded with time and their rates of turnover were calculated. The results demonstrated product–precursor relationships along the pathway of hexosamine synthesis and that of hexuronic acid synthesis. The rates of synthesis of UDP-*N*-acetylhexosamine and UDP-glucuronic acid were $1{\cdot}5 \pm 0{\cdot}3$ and $0{\cdot}24 \pm 0{\cdot}03$ mμmoles/min./g. of tissue respectively. These results indicated the average turnover time of the total tissue glycosaminoglycans to be about 5 days.

Details of the intermediary metabolism of precursors of glycosaminoglycans in tissues actively synthesizing these polymers are sparse. The work of Schiller, Slover & Dorfman (1961) suggested that the skins of rats immediately *post partum* might furnish material capable of carrying out the biosynthesis of glycosaminoglycans at a rate sufficient to make feasible experimental measurements of the flux and turnover of metabolic intermediates. The present paper records the results of a series of experiments on skins from such rats injected intraperitoneally with [U-^{14}C]glucose, from which the intermediates were extracted and separated by chromatography on Dowex 1 (formate form).

MATERIALS AND METHODS

Materials. All sugars and derivatives were of D-configuration unless otherwise stated. NAD, NADP, nucleotide sugars, hexose monophosphates, fructose 1,6-diphosphate and 3-phosphoglyceraldehyde were obtained from C. F. Boehringer und Soehne G.m.b.H. (Mannheim, Germany). Other nucleotides were obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Glucosamine 6-phosphate was prepared by the action of hexokinase on glucosamine as described by Jourdain & Roseman (1962). Some of this product was acetylated (Distler, Merrick & Roseman, 1958) to yield *N*-acetylglucosamine 6-phosphate. [U-^{14}C]Glucose of high specific radioactivity (196 mc/m-mole) was obtained from The Radiochemical Centre (Amersham, Bucks.). 2,5-Bis-(5-*tert.*-butylbenzoxazol-2-yl)thiophen was obtained from Thorn Electronics Ltd. (Tolworth, Surrey). Ion-exchange resins Dowex 50 and Dowex 1 were supplied by V. A. Howe and Co. Ltd. (London, W. 11). Other reagents were supplied by British Drug Houses Ltd. (Poole, Dorset).

Centrifugation. Centrifugation was carried out in an MSE High Speed 18 centrifuge with an 8 × 50 ml. head type 69181.

Measurement of extinction. Extinctions were read on a Hilger Uvispek H700 instrument.

Tissue extraction. Wistar strain rats, less than 24 hr. *post partum*, were injected intraperitoneally with 10 μl. of [U-^{14}C]glucose, containing 10^6 counts/min. in 2 mμmoles, in 0·9% NaCl. The rats were placed in an incubator at 37° for periods of up to 2 hr. At predetermined times the animals were decapitated, and the skins were removed and freeze-clamped at −40°. The time from decapitation to freezing was not longer than 15 sec. The tissue was powdered in a percussion mortar, weighed and extracted with twice its weight of 0·4 M-$HClO_4$. The homogenate was dispersed in a cooled Omnimix (Ivan Sorvall Inc., Norwalk, Conn., U.S.A.) for 45 sec. at full speed and centrifuged for 5 min. at 20000*g*. The pellet was re-extracted twice as above with 0·2 M-$HClO_4$ in the Omnimix for 30 sec. This technique appeared to release at least 98% of the extractable phosphates, as indicated by a further three extractions of the pellet releasing less than 2% more. Replacement of $HClO_4$ with 5% (w/v) trichloroacetic acid gave similar extractions, but use of cold ethanol–water (1:1, v/v) or hot water gave lower yields of extractable phosphates. The combined supernatants were brought to pH 6·8 with 4 N-KOH (monitored with a glass electrode) and were centrifuged for 5 min. at 12000*g*. The temperature throughout the extraction procedure was maintained below 5°.

Separation of components. The whole supernatant fraction, approx. 50 ml. from 7 g. of tissue, was applied to the top of a 20 cm. × 1 cm. column of Dowex 1 (X8; formate form; 200–400 mesh) and eluted as described by Hurlbert, Schmitz, Brumm & Potter (1954). The whole operation was

conducted at 4° at a flow rate of 0·8 ml./min.; 5 ml. fractions were collected and their extinctions read at 260 and 275 mμ. Known samples of glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, 3-phosphoglyceraldehyde, glucosamine 6-phosphate, *N*-acetylglucosamine 6-phosphate, UDP-*N*-acetylglucosamine, UDP-glucose, UDP-glucuronic acid, NAD and NADP were run on the column, and their elution volumes were used to calibrate the chromatography system. When the extracted supernatant was chromatographed, the main nucleotide peaks in addition to those mentioned above were identified as CMP, AMP, GMP, UMP, CDP, ADP, GDP, CTP, UDP, ATP, GTP and UTP on the basis of their u.v. spectra and concomitant phosphate analyses (Chen, Toribara & Warner, 1956). Satisfactory separation of components was obtained with an extract of up to 10 g. of tissue.

Measurement of radioactivity. Samples (0·3 ml.) from all fractions of the column were counted in low-background glass phials containing 8 ml. of scintillation fluid [toluene–2-methoxyethanol (3:2, v/v) containing 80 g. of naphthalene and 4 g. of 2,5-bis-(5-*tert.*-butylbenzoxazol-2-yl)thiophen/l.]. The tubes were counted in a Nuclear–Chicago mark 1 liquid-scintillation system. An external standard was used to correct the counting rate for increased quenching as the eluate concentration increased. Simultaneous measurement of ^{14}C and ^{3}H radioactivity was achieved by suitable discrimination in the above machine.

Recovery of fractions. Pooled fractions containing only formic acid were freeze-dried directly. Those also containing ammonium formate were passed down a 10 cm. × 1·3 cm. column of Dowex 50 (H^+ form; 200–400 mesh), which removed the NH_4^+ ion. Formic acid was partially removed by extracting the fractions three times with an equal volume of diethyl ether. The aqueous phase was then freeze-dried.

Assays. Material that was not retarded on the Dowex 1 (formate form) column was pooled, and a sample was chromatographed on a column (10 cm. × 0·8 cm.) of Dowex 50 (X4; H^+ form; 200–400 mesh). The water eluate from this column was assayed for glucose by the method of Park & Johnson (1949), with glucose oxidase (Huggett & Nixon, 1957) and with anthrone (Yemm & Willis, 1954), and a sample was counted as described above. Where the specific radioactivities of blood glucose and tissue glucose were determined in the same animal, they were found to agree within 3% over times from 20 min. to 2 hr. after injection.

Glucose 6-phosphate and fructose 6-phosphate. These were eluted together from the column, and individual fractions were hydrolysed in 2 N-HCl at 100° for 2 hr. The resulting solutions were vacuum-desiccated and redissolved in a small volume of water. Samples were assayed for hexose by the anthrone reaction, and counted as described above.

Fructose 1,6-diphosphate. The sample containing fructose 1,6-diphosphate was freeze-dried, dissolved in a small volume of water and assayed for fructose (Dische & Borenfreund, 1951) and counted for radioactivity.

UDP-N-acetylhexosamine. Samples corresponding to this material were freeze-dried, hydrolysed for 30 min. at 100° in 0·01 N-HCl and then freeze-dried. This material was dissolved in a small volume of water and chromatographed on a column (3·0 cm. × 0·6 cm.) of Dowex 1 (formate form) to remove UDP and P_i. The water eluate was assayed for *N*-acetylhexosamine by the method of Good & Bessman (1964). A sample of the eluate was deacetylated by heating it in 1 N-HCl in a sealed tube at 100° for 2 hr. The hydrolysate was dried in a vacuum desiccator over NaOH, dissolved in a small volume of water and applied to a column (3·0 cm. × 0·3 cm.) of Dowex 50 (H^+ form). Any neutral sugars were removed with a water wash. The amino sugars were then eluted with 0·5 N-HCl. The eluate was freeze-dried, redissolved and assayed for hexosamine by the method of Blix (1948), and a sample was counted.

Some deacylated samples were separated by chromatography on a thin layer of silica gel G (Mallinckrodt Chemical Works, St Louis, Mo., U.S.A.) in propan-1-ol–aq. ammonia (sp.gr. 0·88) (13:7, v/v). Areas were identified as containing glucosamine and galactosamine by spraying with *p*-anisidine reagent (Hough, Jones & Wadman, 1950) and the corresponding areas on unsprayed duplicates were removed and analysed for each hexosamine by the method of Blix (1948). Still further samples were analysed by gas–liquid chromatography as the trimethylsilyl ethers of the *N*-acetylated methyl glycosides (Clamp, Dawson & Hough, 1967). The glucosamine/galactosamine ratio was used to correct the original *N*-acetylhexosamine assay, allowing for the different colour yields of the two sugars.

UDP-hexose. Samples corresponding to this material were hydrolysed for 5 min. at 100° in 0·01 N-HCl, freeze-dried and chromatographed on Dowex 1 (formate form) as for UDP-*N*-acetylhexosamine. The eluate was assayed for reducing sugar by the method of Park & Johnson (1949) and a sample counted as described above. Further portions were chromatographed on a thin layer (0·25 mm.) of cellulose (Whatman microgranular CC 41) prepared in 0·1 M-sodium phosphate buffer, pH 5·0, the solvent being acetone–butan-1-ol–water (5:4:1, by vol.), and run twice, the plate being dried between the runs. The sugars were located with *p*-anisidine reagent (as above) and identified areas were removed, the sugars eluted with ethanol–4 N-formic acid (49:1, v/v) and their extinctions read at 390 mμ against standards. Samples of these solutions were counted for radioactivity. Further isolated samples were analysed by gas–liquid chromatography as described above.

N-Acetylglucosamine 6-phosphate. The fraction was freeze-dried, dissolved in a small volume of water and assayed for *N*-acetylglucosamine (Good & Bessman, 1964). To obtain the specific radioactivity of a radioactive sample it was necessary to free it of hexose monophosphate. The sample was hydrolysed in 2 N-HCl for 2 hr. at 100° in a sealed tube, vacuum-desiccated, dissolved in a small volume of water and applied to a column (3·0 cm. × 0·3 cm.) of Dowex 50 (H^+ form; 200–400 mesh). This was washed with water, and the glucosamine was eluted with 0·5 N-HCl and freeze-dried. After being dissolved in a small volume of water it was assayed for hexosamine (Blix, 1948) and a sample counted.

UDP-glucuronic acid. Freeze-dried pooled samples were hydrolysed for 15 min. at 100° in 1 N-HCl and the lactone-containing solution was concentrated by freeze-drying. This solution was chromatographed on Dowex 1 (formate form) to remove various anionic products. No uronic acid-reacting material remained on the column. The concentrated water eluate was chromatographed, identified and measured with the thin-layer cellulose system detailed above. Samples of the material from the chromatogram were also counted.

Glycogen. The glycogen was extracted (Walaas & Walaas 1950) and determined by the method of Randle, Newsholme & Garland (1964).

Glycosaminoglycans. Fat-extracted dried skins were incubated with papain, deproteinized, dialysed and treated with cetylpyridinium chloride as in the method of Scott (1960).

Tissue-space experiments. Skins were incubated in a solution containing, in 50ml.: $5{\cdot}6\times10^6$ counts/min. of [U-^{14}C]sucrose (specific radioactivity 9·6mc/m-mole) and 25μc of ^3H_2O. At various times samples of skin were removed, rapidly rinsed in water and blotted gently before weighing. They were then homogenized and the centrifuged supernatants (5000g for 10min.) were analysed for radioactivity.

Determination of blood glucose. In one experiment to

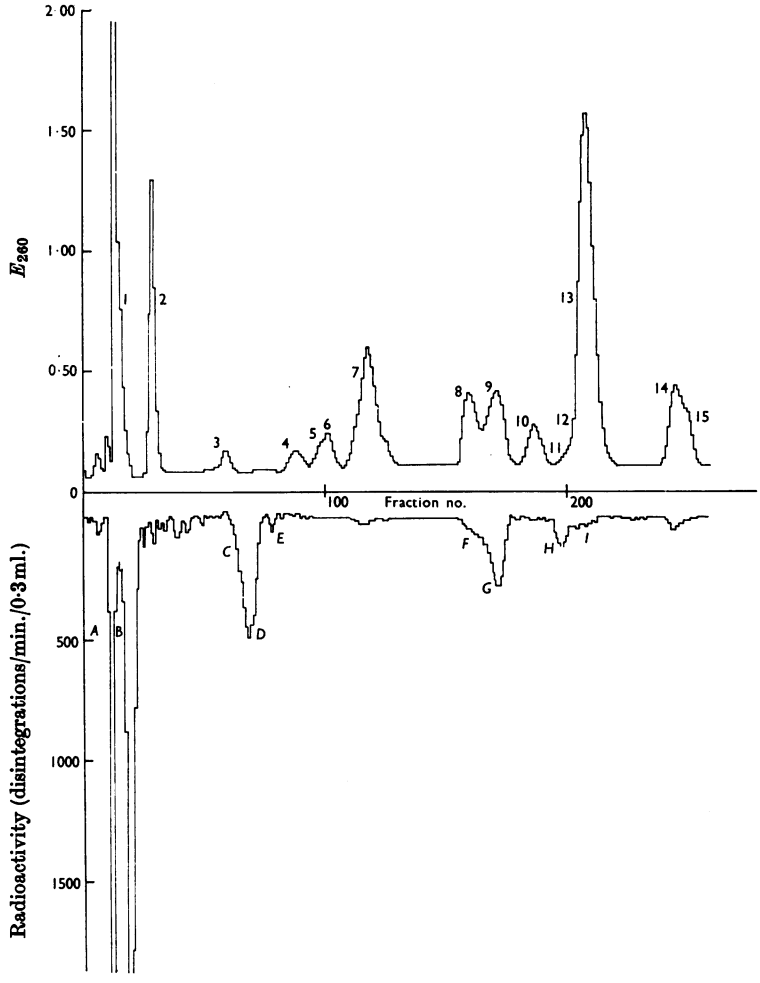


Fig. 1. Elution profile of E_{260} and incorporated radioactivity of the acid-soluble extract of young rat skin 1hr. after the injection of [U-^{14}C]glucose. The extract of 7g. wet wt. of young rat skin was applied to a Dowex 1 (formate form) column as described in the text. Extended gradient elution was carried out by using a 220ml. sealed mixing vessel. The reservoir contained: tubes 0–26, 1N-formic acid; tubes 26–120, 4N-formic acid; tubes 120–174, 4N-formic acid + 0·25M-ammonium formate; tubes 174–205, 4N-formic acid + 0·4M-ammonium formate; tubes 205–250, 4N-formic acid + 0·8M-ammonium formate. A 0·3ml. sample from each fraction was counted as described in the text. Nucleotide peaks: 1, NAD; 2, AMP; 3, NADP; 4, GMP; 5, CDP; 6, UMP; 7, ADP; 8, UDP-*N*-acetylglucosamine and UDP-*N*-acetylgalactosamine; 9, UDP-glucose and UDP-galactose; 10, GDP; 11, CTP; 12, UDP and UDP-glucuronic acid; 13, ATP; 14, GTP; 15, UTP. Radioactivity peaks: *A*, glutamic acid; *B*, lactic acid; *C*, *N*-acetylglucosamine 6-phosphate; *D*, glucose 6-phosphate and fructose 6-phosphate; *E*, 3-phosphoglyceraldehyde; *F*, UDP-*N*-acetylhexosamine; *G*, UDP-hexose; *H*, fructose 1,6-diphosphate; *I*, UDP-glucuronic acid.

determine the time-course of uptake and decay of [U-^{14}C]-glucose in the blood, a group of eight young rats of similar weight (5·3–5·6g.) were decapitated at intervals after intraperitoneal injection of [U-^{14}C]glucose. After injection it was noticeable that some rats showed seepage of liquid out of the point of injection (about one in ten rats); for this experiment any rat in which this was observed was rejected, as it was important to keep the dose of label constant. A blood sample (0·2ml.) from each rat was lysed in water, deproteinized with 5% (w/v) trichloroacetic acid and centrifuged at 5000*g* for 10min. The trichloroacetic acid was removed by extracting each supernatant six times with twice its volume of freshly distilled diethyl ether. The aqueous layer was removed and passed through a column (3cm.×0·3cm.) of mixed-bed Dowex 1 (Cl^- form) and Dowex 50 (Na^+ form) (200–400 mesh) resin to remove anions and cations. The water eluate was assayed for glucose by the methods described above and samples were counted. The results are shown in Figs. 2(*a*) and 2(*b*). Two samples of concentrated eluate were chromatographed on the thin-layer cellulose system described; 1cm. squares from the base line to the solvent front were removed and each was counted in a glass vial with 5ml. of scintillation fluid. More than 95% of the radioactivity was in association with a *p*-anisidine-positive region co-chromatographing with standard glucose.

RESULTS

Fig. 1 shows the typical elution profile of a chromatographic separation performed on an extract of 7g. of rat skin (the rats having been given an injection of [U-^{14}C]glucose 1hr. before decapitation) and records the radioactivity in each fraction.

Variation with time of the specific radioactivity of blood glucose after injection with [U-^{14}C]*glucose.* The size of the young rats precluded the determination of the variation of the specific radioactivity of the blood glucose with time by blood-sampling techniques on one animal. It was necessary to use a group of animals as described above. The experiment was carried out with rats of similar weight and with careful control of the dose of [U-^{14}C]glucose injected, to minimize the effect that variation of body weight and the amount of injected [U-^{14}C]-glucose have on the specific radioactivity of the blood glucose. Fig. 2(*a*) shows the variation of blood glucose specific radioactivity with time, and Fig. 2(*b*) shows the variation of log(specific radioactivity) of blood glucose with time. The latter indicates the decrease in specific radioactivity to be a single-rate process with a half-time of 46min. The general form of Fig. 2(*a*) was assumed to apply to all subsequent experimentally injected animals.

Variation with time of the specific radioactivity of the intermediates in the glycosaminoglycan biosynthetic pathways. The specific radioactivities of the intermediates in the pooled extract of eight to ten rat skins were determined for each of several time-intervals. The effects of animal size and variation in the dose of radioactivity were not minimal in these experiments, but, as indicated by Zilversmit, Entenman & Fischler (1942), the proportions of the specific radioactivities of the intermediates in separate animals would be much more constant

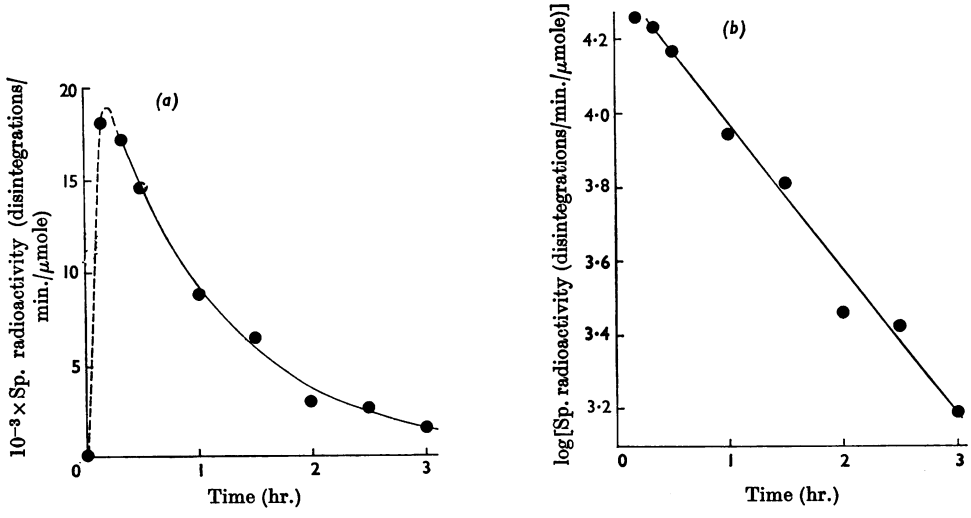


Fig. 2. (*a*) Variation with time of the specific radioactivity of the blood glucose of a young rat after the intraperitoneal injection of [U-^{14}C]glucose. Each point was from one animal. (*b*) Results of (*a*) expressed as the variation of log(specific radioactivity of glucose) with time.

than the specific radioactivities themselves. The specific radioactivities of the intermediates in each experiment were thus recorded as a ratio to that of the tissue glucose determined in the same extract. These ratios were plotted in the time-course of changes in the specific radioactivity of the blood glucose, producing Fig. 3. This normalizes the results from several experiments to one set of conditions and produces a time-course–specific radioactivity curve for each of the following intermediates: hexose monophosphate, fructose 1,6-diphosphate, *N*-acetylglucosamine 6-phosphate, UDP-*N*-acetylhexosamine, UDP-hexose and UDP-glucuronic acid. These results were used to calculate the turnover times of the intermediates as described by Zilversmit *et al.* (1942), and from the tissue contents of the intermediates their rates of synthesis were obtained.

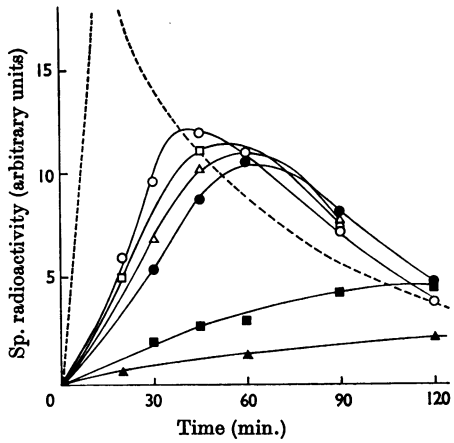


Fig. 3. Ratio of the specific radioactivities of intermediates, extracted from young rat skin after intraperitoneal injection of [U-^{14}C]glucose, plotted on the time-course of change in the specific radioactivity of the blood glucose (Fig. 2*a*). ○, Hexose monophosphate; □, *N*-acetylglucosamine 6-phosphate; △, fructose 1,6-diphosphate; ■, UDP-hexose; ●, UDP-*N*-acetylhexosamine; ▲, UDP-glucuronic acid; ----, glucose.

Product–precursor relationships. To treat the data by this method, it was assumed that there was a direct product–precursor relationship between UDP-glucose and hexose monophosphate, between UDP-glucuronic acid and UDP-glucose, between UDP-*N*-acetylglucosamine and *N*-acetylglucosamine 6-phosphate, and between *N*-acetylglucosamine 6-phosphate and the hexose monophosphate pool. As a direct product–precursor relationship implies conversion of precursor into product without the occurrence of a stable intermediate, the latter two statements require some justification. In the hexosamine pathway from fructose 6-phosphate to UDP-*N*-acetylglucosamine there are three other intermediates, but only one of them, *N*-acetylglucosamine 6-phosphate, was easily isolated and counted. Glucosamine 6-phosphate was not identified on ion-exchange chromatography in the region in which standard glucosamine 6-phosphate was eluted. This suggested that it was perhaps present at a concentration too low to be detected by this system. However, if it was present at a low concentration compared with other intermediates, it would have little effect on the passage of label from fructose 6-phosphate to *N*-acetylglucosamine 6-phosphate. The calculation of the rate of *N*-acetylglucosamine 6-phosphate synthesis was made under this assumption. *N*-Acetylglucosamine 1-phosphate was also not isolated. However, if it is assumed that the steady-state equilibrium of the enzyme acetylglucosamine phosphomutase (EC 2.7.5.2) differs little from the free equilibrium, i.e. 14% of *N*-acetylglucosamine 1-phosphate (Reissig, 1956), the time-course–specific radioactivity curve of *N*-acetylglucosamine 1-phosphate will be close to that of

Table 1. *Tissue contents and turnover rates of intermediates in the biosynthesis of glycosaminoglycans in young rat skin*

Tissue contents are given as means ± S.D. of five to eight experiments.

	Tissue content (*a*) (mμmoles/g. wet wt.)	Concn. in cellular water (mM)	Turnover time (*b*) (min.)	Turnover rate (*a*/*b*) (mμmoles/g. wet wt./min.)
Hexose monophosphate	250±18	0·72	27·2±0·9	9·2±1·0
Fructose 1,6-diphosphate	100±8	0·29	14·3±0·5	7·0±0·8
N-Acetylglucosamine 6-phosphate	8±2	0·02	4·0±0·3	(2·0±0·3)
UDP-*N*-acetylglucosamine UDP-*N*-acetylgalactosamine	76 38 } 114±6	0·22 0·11	76·0±9·0	1·5±0·3
UDP-glucose UDP-galactose	78 26 } 104±8	0·21 0·07	18·6±1·3	5·6±0·6
UDP-glucuronic acid	28±2	0·08	117±8	0·24±0·03

N-acetylglucosamine 6-phosphate. Under these conditions it can be calculated that the error in the rate of UDP-*N*-acetylhexosamine synthesis is no greater than 4%. It is also possible that the rate of exchange between the two forms of hexosamine phosphate is large compared with the flow along the pathway, thus tending to equalize their specific radioactivities. The calculated rate of synthesis of UDP-*N*-acetylhexosamine is therefore unlikely to be greatly in error as a result of this assumption. The results of this analysis are given in Table 1. The determination of the rate of fructose 1,6-diphosphate synthesis was used in an attempt to find how much material was leaving the hexose monophosphate pool in the direction of glycolysis. It will be seen that the total flux of material passing into UDP-hexose, UDP-*N*-acetylhexosamine and fructose diphosphate is greatly in excess (approx. 50%) of the rate of synthesis of hexose monophosphate. This point is elaborated in the Discussion section.

Tissue-space experiments. These were undertaken to determine the total tissue-water volume, extracellular-water volume, and thus by difference the cell-water volume. Tritiated water was equilibrated in a compartment amounting to 71% of the wet tissue bulk, whereas desiccation revealed a total tissue-water space of 85%. The sucrose-accessible space was measured as 36% of the wet weight. From these values it is possible to determine the cell-water volume as 35% of the wet weight. This was used for computing the concentration of metabolites as they occur within the cell (Table 1).

Identity of UDP-uronic acid. On thin-layer chromatography as described the isolated hexuronic acid ran as a single spot with the same R_F as standard glucuronolactone; however, no iduronic acid was available for comparison. On the basis of this and the carbazole/orcinol ratio it was identified as glucuronic acid (Bitter & Muir, 1962; Khym & Doherty, 1952).

DISCUSSION

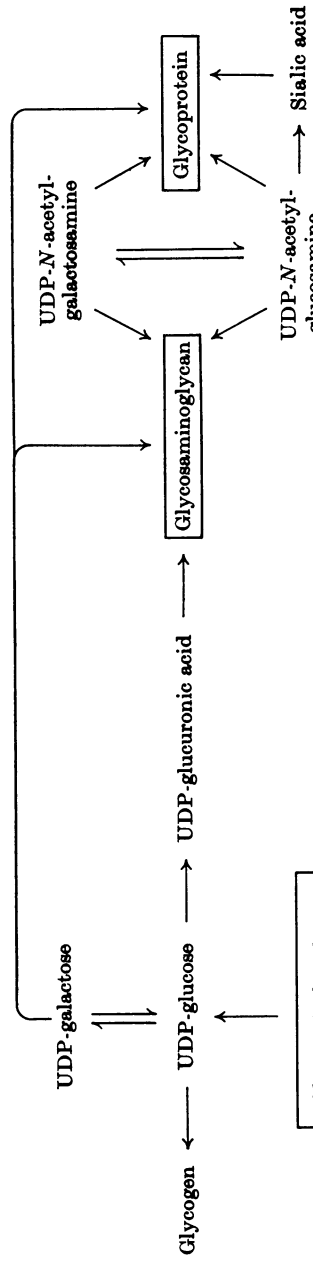
The object of the work reported in this paper was to establish the tissue content and turnover times of intermediates primarily involved in glycosaminoglycan biosynthesis. From this information it would be possible to predict a rate of synthesis and a turnover time for the polymers produced in rat skin.

Previous work on the biosynthesis of hyaluronic acid in cell-free systems involved the use of those intermediates assumed to be immediately before the polymerizing reaction (Glaser & Brown, 1955; Markovitz, Cifonelli & Dorfman, 1959; Schiller *et al.* 1961; Schiller, 1964). Thus Schiller (1964), using a particulate fraction of homogenate of young rat skin, demonstrated hyaluronate synthetase activity by the incorporation of radioactivity from UDP-*N*[^{3}H]-acetylglucosamine into hyaluronic acid. However, the low activity observed, 0·14mμmole of monosaccharide incorporated/hr./g. of tissue, reflected little of the tissue's ability to form polymer *in vivo*. An explanation of this resides in the suggestion that the synthesizing ability of such tissues may need the structural integrity of the cellular apparatus.

In the present study the flow of ^{14}C through the intermediates is in agreement with the pathway as shown in Scheme 1, but raises some problems. Reference to Table 1 shows that the calculated total flux out of the hexose monophosphate pool is greater than the flux into it. In some glycogen-synthesizing tissues (ascites-tumour cells, rat diaphragm) it has been shown that a disproportionate amount of ^{14}C from [U-^{14}C]glucose is incorporated into UDP-glucose compared with that in the hexose monophosphate pool and the glycolytic intermediates (Sims & Landau, 1965). This tissue contained 0·6mg. of glycogen/g. wet wt. (average of three results: 0·58, 0·61, 0·61), and the rapid incorporation of ^{14}C from [U-^{14}C]glucose (the specific radioactivity after 30min. was 1064 disintegrations/min./μmole of hexose unit) indicated an appreciable rate of glycogen synthesis. Possible explanations for the discrepancies, as indicated by Sims & Landau (1965), are: within one cell there may be two pools of hexose monophosphate, one committed to UDP-glucose synthesis and one to glycolysis, as, for instance, the rapid conversion of glucose 1-phosphate into UDP-glucose near to the site of hexokinase action on glucose, thus preventing complete equilibration of hexose monophosphate with the bulk cell pool; there may be more than one type of cell, one synthesizing glycogen at a low glycolytic rate and one glycolysing at a fast rate and not synthesizing glycogen. The calculated rate of UDP-hexose synthesis may thus be falsely high, as the true specific radioactivity of its hexose monophosphate precursor may have been higher than that measured. The calculated rate for UDP-glucuronic acid would also be affected if there were a similar disparity between separate UDP-hexose pools.

A further point that requires elucidation concerns the measurements of the amounts and specific radioactivities of UDP-*N*-acetylglucosamine and UDP-*N*-acetylgalactosamine and of UDP-glucose and UDP-galactose (Table 2). These show that both epimers of each pair of nucleotide sugars has equal specific activity, suggesting that rapid epimerization occurs. Thus synthesis of UDP-glucose can be considered as synthesis directly into a UDP-hexose pool and synthesis of UDP-*N*-acetylglucosamine similarly into a UDP-hexosamine pool.

The rates of synthesis recorded for the hexosamine



Scheme 1. Pathway of biosynthesis of glycosaminoglycans and some related polymers. Arrows indicate direction of flow only.

Table 2. *Specific radioactivities of glucose, galactose, N-acetylglucosamine and N-acetylgalactosamine isolated from their UDP derivatives*

The compounds isolated from their UDP derivatives were separated on thin-layer plates (0·25mm.) of cellulose with the solvent acetone–butan-1-ol–water (5:4:1, by vol.). Three other samples from different time-course experiments were separated, and by using thin layers of silica gel G and propan-1-ol–aq. ammonia (sp.gr.0·88) (13:7, v/v) some deacylated hexosamine samples were also separated. Specific radioactivities of the pairs differed by less than 10% in each case. Gas–liquid chromatography confirmed the proportions of components as found by thin-layer chromatography.

	R_F	Hexose (μmole)	Sp. radioactivity (counts/min./ μmole)
Glucose	0·31	0·097	10000
Galactose	0·26	0·036	9400
N-Acetylglucosamine	0·53	0·079	5100
N-Acetylgalactosamine	0·46	0·032	4700

pathway probably represent synthesis into glycoprotein and glycosaminoglycans, whereas the substantially lower rates of synthesis of the UDP-glucuronic acid pathway are destined almost solely for glycosaminoglycan synthesis. The tissue content of glycosaminoglycans was found to be approx. 0·98mg./g. wet wt., corresponding to 0·36mg./g. of polymer uronic acid. If all the uronic acid were involved in the synthesis of this material, its average turnover time would be 5 days. Preliminary separation of labelled polymer by fractional solubility of their cetylpyridinium chloride complexes on cellulose columns (Antonopoulos, Borelius, Gardell, Hamnstrom & Scott, 1961) indicates this to be composed of hyaluronic acid (about 60%), turning over much faster than the sulphated glycosaminoglycan fractions.

L-Glutamine–D-fructose 6-phosphate aminotransferase (EC 2.6.1.16), the first step in the synthesis of UDP-*N*-acetylhexosamine, has been shown by Kornfeld, Kornfeld, Neufeld & O'Brien (1964) in a preparation from rat liver to be subject to end-product inhibition by various UDP-sugars. If it is assumed that the skin contents of UDP-*N*-acetylglucosamine, UDP-*N*-acetylgalactosamine, UDP-glucose, UDP-galactose and UDP-glucuronic acid are evenly distributed in the cell water, it can only be concluded that this enzyme must be heavily inhibited, and that the observed rate represents only a small part of its potential activity. However, the enzyme in skin may have different properties from that in liver, or some as yet unrecorded activator may be present.

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