Alterations in Glycosaminoglycan Metabolism in β -Aminopropionitrile-Treated Chick Embryos

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1. $\text{Na}_2^{35}\text{SO}_4$, $[1-^{14}\text{C}]\text{glucosamine}$ and $[1-^{14}\text{C}]\text{acetate}$ were used as precursors of the sulphated glycosaminoglycans to study the biochemical effect of β -aminopropionitrile in chick embryos. The incorporation of all three precursors was decreased in the treated embryos between days 7 and 10 of embryonic development. No inhibition of incorporation of these precursors occurred between days 16 and 20 of embryonic development at the dosages of β -aminopropionitrile used. 2. β -Aminopropionitrile treatment also decreased the amount of *N*-acetylhexosamines in the chick embryo and decreased the percentage of the hexosamine esterified by nucleotides. Respiration was decreased by homogenates prepared from treated embryos. Likewise, UDP-xylosyl- and UDP-galactosyl-transferase activities were decreased in treated embryos and cartilage from embryos and growing chicks. 3. The data suggest that β -aminopropionitrile, in addition to the known lathyrogenic activity, either is or gives rise to a potent metabolic poison that interferes with basic cellular metabolism. The results are consistent with a decreased rate of ATP generation as an explanation for the decrease in glycosaminoglycan synthesis.

Lathyrisms is an experimental syndrome that occurs after eating the seeds of certain sweet peas (genus *Lathyrus*). In the classic experiment in the rodent, *Lathyrus odoratus* seeds were fed to young rats and growth failure, crooked spines, crooked long bones and rib deformities were consistently present in the majority of animals (Geiger *et al.*, 1933). The toxic material in *Lathyrus odoratus* was shown to be the nitrile β -aminopropionitrile by Schilling & Strong (1954).

Structural and biochemical alterations of the major components of connective tissue have been demonstrated in a variety of avian and mammalian species by feeding or injecting nitriles (Tanzer, 1965; Piez, 1968). Levene & Gross (1959) suggested that the increased solubility of collagen was due to defective intramolecular cross-linkage in the collagen molecule that altered molecular aggregation in the extracellular tissue. Inhibition of cross-linkage biosynthesis by β -aminopropionitrile has been demonstrated for both elastin (Miller *et al.*, 1965; O'Dell *et al.*, 1966) and collagen (Siegel & Martin, 1970). Concentrations of β -aminopropionitrile that do not inhibit protein synthesis markedly depress the cross-linkage enzyme lysyl oxidase (Siegel & Martin, 1970).

A specific alteration of the third major component of connective tissue, the glycosaminoglycans, by nitriles has not been shown at dosages that decrease

the cross-linkages in collagen and elastin. By using higher dosages Castellani *et al.* (1959) demonstrated significant depressions in the galactosamine content of lathyritic cartilage, and lathyrogens administered *in vivo* appear to decrease uniformly glycosaminoglycan content (Rosmus *et al.*, 1966).

A change in glycosaminoglycan metabolism could not be demonstrated in chick embryo cartilage (Levene *et al.*, 1966). Embryos were injected on day 14 of incubation with 5 mg of β -aminopropionitrile per egg and both proteoglycans and total glycosaminoglycans were isolated, purified and characterized from embryos killed 2, 4 and 6 days later. Neither quantitative nor qualitative alterations were found in the glycosaminoglycans although a marked increase in extractable collagen occurred. However, larger amounts of β -aminopropionitrile in embryos of the same age have been reported to decrease the number of glycosaminoglycan granules in the hypertrophic zone of the cartilage (Matukas *et al.*, 1967) and to increase the solubility of protein-polysaccharide complexes in 1 M-NaCl (Glimcher *et al.*, 1969).

The aim of the present study was to define a system in which inhibition of glycosaminoglycan synthesis could be shown. This was accomplished by using chick embryos and by measuring the incorporation of sulphate, hexosamine and acetate into isolated glycosaminoglycan, the concentrations of esterified

and non-esterified *N*-acetylhexosamines in the soluble cell pool and the activities of the cartilage glycosyltransferases.

Experimental Procedures

Materials

Fertile eggs from a cross between Cornish roosters and Hubbard hens were given by the Keith Smith Hatchery, Hot Springs, Ark., U.S.A. Chondroitin sulphate and papain were obtained from Sigma Chemical Company, St. Louis, Mo., U.S.A. β -Aminopropionitrile (HCl or fumarate) and cetylpyridinium chloride were obtained from Calbiochem, Los Angeles, Calif., U.S.A. Carrier-free $\text{Na}_2^{35}\text{SO}_4$, sodium $[1\text{-}^{14}\text{C}]\text{acetate}$ (54 Ci/mol), D- $[1\text{-}^{14}\text{C}]\text{glucosamine hydrochloride}$ (1.4 Ci/mol), UDP- $[\text{U-}^{14}\text{C}]\text{galactose}$ (280 Ci/mol) and UDP- $[\text{U-}^{14}\text{C}]\text{xylose}$ (172 Ci/mol) were obtained from New England Nuclear Corporation, Boston, Mass., U.S.A.

All chemicals were reagent grade and were obtained from the Fisher Scientific Company, St. Louis, Mo., U.S.A.

Incubation procedure

Fertile eggs were incubated at 37°C with high humidity and the eggs were rotated daily. Injections were made on to the chorioallantoic membrane. The substance to be injected was dissolved in sterile 0.9% saline (0.1–0.2 ml) and adjusted to pH 7.0–7.4. Since the mortality due to β -aminopropionitrile is significant, care was taken to ensure that only living embryos were analysed. Visual evidence of heart beat was the criterion used to determine if the embryos were alive. When young chicks were used the birds were given either powdered Purina chick diet or the same diet with added 0.2% β -aminopropionitrile (as the fumarate) from the day of hatching.

Glycosaminoglycan methods

Embryos or epiphyseal cartilage slices were weighed and homogenized in 2 ml of 0.9% saline containing 10 mg of carrier chondroitin sulphate. The glycosaminoglycans were isolated and partially purified as described by Perlman *et al.* (1964). The final precipitate was weighed, dissolved and 0.1 ml was taken for radioactivity counting. The uronate content of each sample was determined by the carbazole method of Dische (1947). The ^{35}S or ^{14}C incorporation was then expressed as c.p.m./ μg of glucuronate.

The carrier added was approximately a 10-fold excess of the glycosaminoglycan present in one chick embryo. The glucuronate determination on the isolated material allows an assessment of losses

during isolation. Recovery of the added chondroitin sulphate on this basis was usually about 70%. When the radioactivity is expressed on the basis of the glucuronate present in the isolated material, a measure of the extent of radioisotope incorporation is obtained. The alkali step was included first to liberate the carbohydrate chains from the protein-polysaccharide complex and to allow rapid equilibration with the carrier.

All counting for radioactivity was done in a Packard Tri-Carb liquid-scintillation spectrometer by using 10 ml of the xylene-dioxan-Cellosolve scintillation mixture described by Bruno & Christian (1961).

Measurement of *N*-acetylhexosamines

Embryos were homogenized in water and protein was precipitated by 10% trichloroacetic acid. The precipitate was washed once with 10% trichloroacetic acid and the washing was added to the first extract. This solution was then extracted three times with diethyl ether to remove the acid. The aqueous phase was freeze-dried and dissolved in water (1 ml); the pH was then adjusted to 7.0. *N*-Acetylhexosamines were determined by the Reissig *et al.* (1955) modification of the Morgan-Elson reaction before and after dilute acid hydrolysis to differentiate between esterified and non-esterified *N*-acetylhexosamines (Strominger, 1957). The esterified *N*-acetylhexosamines are predominantly UDP-*N*-acetylhexosamines. The values are expressed in μg of *N*-acetylglucosamine/mg wet wt. of embryo.

Assay of glycosyltransferases

The transferase activities were measured as described by Grebner *et al.* (1966) with minor modifications. Whole embryos, epiphyseal cartilage slices or embryonic cartilage samples were chilled, then homogenized in 0.2 M-sucrose and 0.05 M-Tris-acetate buffer, pH 7.0. The homogenate was centrifuged at 10000g for 10 min and the supernatant fluid was used without further purification. Xylosyltransferase activity was assayed at pH 6.5 with $3.2\mu\text{M}$ -UDP- $[\text{U-}^{14}\text{C}]\text{xylose}$ and 6 mM-MnCl₂; galactosyltransferase activity was assayed at pH 5.5 with $7.4\mu\text{M}$ -UDP- $[\text{U-}^{14}\text{C}]\text{galactose}$ and 4 mM-MnCl₂. After incubation of the enzyme preparation with the radioactive substrates an equal volume of cold 10% (w/v) trichloroacetic acid was added to stop the reaction. The precipitate was left for 30 min in the cold, then collected by centrifugation and washed three times with 5% (w/v) trichloroacetic acid and once with ethanol-diethyl ether (1:1, v/v). The residue was dried and dissolved in 0.1 ml of 1 M-KOH, then this solution was diluted 5-fold. Portions were assayed for radioactivity and protein. Boiled enzyme was used as a control.

O₂-uptake measurements

O₂ uptake was measured with a Gilson respirometer. The embryo homogenates were prepared in 0.2M-sucrose and 0.5ml of a 10% (w/v) homogenate was added to a 25ml respiration flask. The final incubation medium of 2ml contained 0.05M-sucrose, 0.01M-potassium phosphate buffer, pH7.4, 8mM-MgCl₂, 1mM-ATP, 30mM-glucose, 2.5mM-potassium pyruvate and 2.5mM-potassium fumarate. After a 15min equilibration period before addition of the substrates, O₂ uptake was recorded each 10min for 90min and the values were calculated from the first 60min, where the rate was linear.

Results

³⁵SO₄ incorporation into chick embryo

To establish the day of maximum sulphate incorporation into the whole embryo, 5μCi of carrier-free Na₂³⁵SO₄ was injected after 5 days of incubation 24h before the study. The homogenates were dialysed against 0.15M-Na₂SO₄ overnight, then dialysed against water for 2h and digested in 0.1M-NaOH for 18–24h. After digestion 0.5ml was taken for ³⁵S determination. The incorporation by embryos of different age expressed as c.p.m./mg wet wt. of the embryo is shown in Fig. 1. Maximum incorporation of sulphate into the whole embryo occurs on day 7 of incubation. The incorporation of the injected ³⁵S per unit of body wt. on day 16 was less than 10% of that incorporated on day 7. The average total incorporation per embryo was 945000 c.p.m. (embryo = 0.79g) on day 7 compared with

Fig. 1. Effect of age on incorporation of ³⁵S into the chick embryo

Embryos were injected 24h before study with 5μCi of Na₂³⁵SO₄ between days 6 and 16 of incubation. The data represent the c.p.m./mg wet wt. of embryo after homogenization and extensive dialysis in 0.15M-Na₂SO₄.

420000c.p.m. (embryo = 16.5g) on day 16. The c.p.m. values are corrected for decay.

The peak time of incorporation of ³⁵S after injection is shown in Fig. 2. Eggs were injected on day 7 of incubation and sulphate incorporation was measured periodically between 30min and 72h after injection. Maximum sulphate incorporation/mg of tissue is attained by 16h with little change in total radioactivity between 24 and 72h. Because the period of maximum sulphate incorporation was from day 7 to day 10 and maximum incorporation occurred between 16 and 72h, the protocol for the β-aminopropionitrile studies was to inject 7-day embryos with the precursor and lathyrogen and kill the embryos 72h after injection.

Effect of β-aminopropionitrile on growth of embryos

Embryos were injected with 3, 6 and 9mg of β-aminopropionitrile on day 7 and were harvested on day 10. The mean wet wt. of controls was 2.0g/embryo; when embryos were injected with 3mg this value was 1.5g/embryo; with 6mg, 1.6g/embryo; and with 9mg, 1.1g/embryo.

Age of embryo and β-aminopropionitrile effect on ³⁵S incorporation

The effect of the nitrile on sulphate incorporation at different embryonic ages was studied by injection on incubation days 7, 10, 12, 15 and 17 with 3mg of β-aminopropionitrile and 5μCi of carrier-free Na₂³⁵SO₄. Controls were injected at the same time with 0.9% NaCl plus 5μCi of Na₂³⁵SO₄. Embryos were harvested 72h after the injection on days 10, 13, 15, 17 and 20. Sulphate incorporation into the whole embryo was studied in 10-day and 13-day embryos. In the 15-, 17- and 20-day embryos epiphyseal cartilage from the long bones was used because

Fig. 2. Sulphate incorporation by 7-day embryos after injection with 5μCi of Na₂³⁵SO₄

skeletal calcification prevented adequate homogenization of the whole embryo. The ^{35}S content of the embryo was determined on the homogenate as described above. The cartilage was weighed, soaked for 8 h in $2\text{M-Na}_2\text{SO}_4$ and washed repeatedly with water. The washed cartilage was digested in conc. $\text{HNO}_3\text{--HClO}_4$ (5:1, v/v). Samples were taken to near dryness and made up to 10 ml with 0.1M-NaOH and 0.5ml was taken for radioactivity counting. Comparison of the nitrile-treated embryos with the controls (Table 1) shows a marked inhibition of ^{35}S incorporation when the nitrile was injected before day 12, with much less inhibition occurring in embryos injected on day 14 and no inhibition occurring in the group injected on day 17.

Effect of β -aminopropionitrile on glycosaminoglycan turnover in lathyrctic cartilage

The effect of the nitrile on sulphate incorporation and turnover was determined by placing two groups of 20 1-day-old chicks on identical diets with one group receiving 0.2% β -aminopropionitrile for 5 days. At the end of 5 days each chick was injected with $25\mu\text{Ci}$ of ^{35}S and killed between 2 and 96 h later. The epiphyseal cartilage was removed and the glycosaminoglycans isolated as described above. Maximum incorporation occurred between 8 and 16 h after injection. The calculated half-life of the ^{35}S for chicks on the control diet was 24 h compared with 35 h for those chicks on the β -aminopropionitrile-containing diet (Fig. 3). These studies suggest that the turnover of sulphate-labelled glycosaminoglycans in the nitrile-treated birds is normal or slightly decreased.

Inhibition of acetate, glucosamine and sulphate incorporation by β -aminopropionitrile

The effect of nitrile on the incorporation of three radioactive precursors (glucosamine, Na_2SO_4 and sodium acetate) into isolated glycosaminoglycans was then studied with up to 12mg of β -amino-

propionitrile. The substrates and nitrile were injected simultaneously on day 7 of incubation and the embryos were studied on day 10. Ten embryos were used at each dose of nitrile. Glycosaminoglycan synthesis was measured by the relative specific radioactivity (carrier plus endogenous glycan, see 'Glycosaminoglycan methods') for each of these precursors, on the basis of hexuronate content in the isolated purified glycosaminoglycan. These values were calculated as c.p.m./ μg of glucuronate, then averaged, and are shown in Fig. 4 relative to the control mean, which was assigned the value of 100% .

Fig. 3. Effect of β -aminopropionitrile on glucosamine, SO_4^- and acetate incorporation into glycosaminoglycan

Embryos were injected on day 7 of incubation with either $5\mu\text{Ci}$ of $\text{Na}_2^{35}\text{SO}_4$ ($\circ$), $1\mu\text{Ci}$ of $[1\text{-}^{14}\text{C}]\text{-glucosamine}$ ($\bullet$) or $2\mu\text{Ci}$ of sodium $[1\text{-}^{14}\text{C}]\text{acetate}$ (Δ) and killed 72 h later. The embryos were homogenized in saline and the glycosaminoglycans isolated with the aid of carrier chondroitin sulphate as described in the text. Values are the percentage of the mean control incorporation calculated in c.p.m./ μg of glucuronate.

Table 1. Age of the embryo and β -aminopropionitrile effect on ^{35}S incorporation into whole embryo or cartilage

Incorporation was determined by measuring non-diffusible ^{35}S in whole-embryo homogenates in the groups injected on days 7 and 10 of incubation. Incorporation into epiphyseal cartilage was measured in the remaining three groups.

	Age (days)		Incorporation (c.p.m./mg of wet wt.)		
	At injection	At time of study	Control	Nitrile (3 mg)	% of control
Whole embryo	7	10	1356	603	44
	10	13	1470	693	47
Cartilage	12	15	528	124	23
	14	17	529	393	74
	17	20	301	285	94

Fig. 4. Effect of β -aminopropionitrile treatment on the turnover of ^{35}S in cartilage

Chicks (1-day-old) were injected with $25\mu\text{Ci}$ of $^{35}\text{SO}_4$ and fed on a Purina chow diet (○) or the same diet with 0.2% β -aminopropionitrile fumarate added (●). The chicks were killed at the indicated time-intervals and cartilage was removed for the isolation of the glycosaminoglycans. The calculated half-life of the ^{35}S is 24 h in the control group and 35 h in the nitrile-treated group.

β -Aminopropionitrile suppressed the incorporation of all three precursors by approximately the same amount. The dose of 3mg/embryo suppressed incorporation by about 50%.

Inhibition of synthesis and esterification of N-acetylhexosamines by β -aminopropionitrile

Because both acetate and glucosamine incorporation into the isolated glycosaminoglycans were depressed by the nitrile, the effect of the same doses of the nitrile on the total and esterified *N*-acetylhexosamines at day 7 of incubation was measured in the embryos. The results of this experiment are shown in Fig. 5. Increased doses depressed the content of trichloroacetic acid-soluble *N*-acetylhexosamines and also increased the percentage of non-esterified *N*-acetylhexosamines from 30% in the control to 90% in the embryos receiving 12mg of β -aminopropionitrile.

Failure of glutamine and glucosamine to alter β -aminopropionitrile inhibition

An attempt was made to reverse the nitrile inhibition of sulphate incorporation into 7-day chick embryos by injecting glutamine and glucosamine.

Fig. 5. Effect of β -aminopropionitrile on *N*-acetylhexosamine concentration and on the percentage of the total *N*-acetylhexosamine that is non-esterified (not UDP-bound)

Chick embryos were injected on day 7 with the indicated amounts of nitrile. After 72h the embryos were killed and homogenized and total *N*-acetylhexosamines were determined as described in the Experimental Procedures section. Each point represents the average of three pooled groups of three embryos each. ●, *N*-Acetylhexosamine concn. ($\mu\text{g/g}$); ○, % of non-esterified *N*-acetylhexosamine.

Substrate amounts (10mg) of these precursors for *N*-acetylhexosamine synthesis were injected with 6mg of β -aminopropionitrile and $5\mu\text{Ci}$ of $\text{Na}_2^{35}\text{SO}_4$ on day 7 of incubation. The radioactivity was measured in the isolated glycosaminoglycan 72h later. The depression of the mean ^{35}S incorporation by nitrile in the control group (receiving neither glucosamine nor glutamine) was comparable with the data in Fig. 3. Neither glucosamine nor glutamine injection altered the inhibition of ^{35}S incorporation by β -aminopropionitrile with the amounts of substrate injected into these 7-day embryos. The effect of the combination of these two substrates on *N*-acetylhexosamine synthesis was not studied.

β -Aminopropionitrile and UDP-glycosyltransferase activities

Two enzymes involved in forming the protein-polysaccharide linkage region of the glycosaminoglycans were assayed in extracts from nitrile-treated and control embryos by using the endogenous acceptors present in the preparations.

Eggs were injected with neutral saline or nitrile (3mg/egg) on days 7, 8 and 11 of incubation and studied 72h later. The enzymes were assayed as described in the Experimental Procedures section. Pelvic rudiments from four embryos were pooled and homogenized for each enzyme preparation. The data in Table 2 represent the average values for duplicate assays on two enzyme preparations. The nitrile

Table 2. *UDP-xylosyl- and UDP-galactosyl-transferase activity in cartilage after β -aminopropionitrile injection*

The enzyme was prepared by homogenizing whole embryos or the proximal ends of the long bones in 0.2M-sucrose buffered with 0.05M-Tris-acetate, pH7.0, 2ml/g wet wt. The dose of nitrile in the treated group was 3mg injected on to the chorioallantoic membrane. The supernatant from this homogenate was prepared by sedimentation at 10000g for 10min. Each assay tube contained 0.1ml of enzyme with either 4.6 μ M-UDP-[14 C]xylose and 6.0mM-MnCl₂ or 8.16 μ M-UDP-[14 C]galactose and 4.0mM-MnCl₂ in a final volume of 0.25ml. The reaction was stopped after incubation for 30min at 37°C.

Age (days)		[14 C]Xylose (c.p.m./mg of protein)		[14 C]Galactose (c.p.m./mg of protein)	
At injection	At time of study	Control	Nitrile	Control	Nitrile
7	10	14400	0	43800	8400
8	11	22500	1800	88000	6300
11	15	21500	16400	85700	78000

Table 3. *Effect of β -aminopropionitrile on transferase activities in vitro*

The UDP-glycosyltransferase enzymes were assayed in cartilage from 11-day chick embryos as described in the text with the nitrile added to the assay mixtures in the concentrations indicated.

β -Aminopropionitrile (mM)	% of activity without nitrile	
	UDP-xylose	UDP-galactose
1.0	81	
2.5	91	102
5.0	79	97
10.0	89	85
50.0	64	76

caused a striking depression of the transferase activities in cartilage from embryos injected on both days 7 and 8. Embryos injected on day 11 were not affected nearly so much as the younger embryos.

These data were confirmed in cartilage-enzyme preparations from chicks that were given the nitrile after hatching. The activities of both UDP-xylosyl-transferase and UDP-galactosyltransferase were depressed by approx. 50% after feeding β -aminopropionitrile for 5 days. On the other hand, when pelvic rudiments from day-12 embryos were incubated in tissue culture for 4 or 16h with nitrile there was no inhibition of transferase activities. Likewise, the nitrile did not inhibit activity significantly when added directly to the enzyme preparation (Table 3). Depression of the transferase activity by nitrile administered *in vivo* could be due to a decrease in the amount of endogenous acceptors for the xylose and galactose rather than to a decrease in enzyme protein.

Effect of β -aminopropionitrile on O₂ consumption

The effect of the nitrile on O₂ uptake was studied in homogenates from nitrile-treated embryos and by adding the nitrile to homogenates for normal embryos. Embryos for the experiment *in vivo* were injected on day 7 with 3mg/embryo and the controls were injected with saline. For the experiments *in vitro* 0.05mM- β -aminopropionitrile was added to the incubation flask at the time of study. The mean O₂ uptake by homogenates (duplicate determinations) from three individual control embryos was 4.0 μ l/h per mg of protein (range 3.3–4.5) and by homogenates from β -aminopropionitrile-treated embryos 0.9 μ l/h per mg of protein (range 0.1–1.6). The depressed uptake when injected *in vivo* did not occur when the nitrile was added to the homogenate *in vitro*, and O₂ uptake was increased to 5.6 μ l/h per mg of protein (range 4.9–7.8).

Discussion

The present results clearly indicate that incorporation of three different precursors into glycosaminoglycans are decreased to approximately the same extent. It is unlikely that the pool sizes of each precursor were altered identically to produce such similar results; hence we interpret this decrease as a decreased synthesis. Schryver (1966) has reported similar findings by studying the tibiotarsi in organ culture from 9-day chick embryos. A concentration of 3.16mM-methyleaminoacetoneitrile added to organ-culture media decreased the total chondroitin sulphate present in the media plus cells when compared with control cultures.

Although the doses we used in this investigation are comparable with those used by Levene *et al.* (1966), we do not feel this investigation conflicts with their report that β -aminopropionitrile does not alter glycosaminoglycan concentrations in cartilage

from older embryos. In their experiments embryos were injected on day 14 and harvested between days 16 and 20, at a time when sulphate incorporation and presumably glycan biosynthesis have decreased to a minimum value just before hatching.

The present results clearly emphasize the importance of the stage of development of the embryo in assessing the effects of nitriles. Sulphate incorporation and UDP-glycosyltransferase activities were not materially affected in the embryo beyond day 15 of development at the dosages used. A maximum rate of incorporation of sulphate into the whole embryo at 7 days of incubation agrees with the maximum rate of uptake of sulphate by chick tibia in culture (Layton, 1950), ^{35}S uptake by radioautography (Amprino, 1955) and the marked increased in embryo cartilage chondroitin sulphate content on day 7 (Huffer, 1970). Larger doses of lathyrogen might produce an effect in the older embryos and we have demonstrated a decrease in transferase activities in chicks that were given nitrile after hatching. However, toxicity of β -aminopropionitrile, even at 6–12mg/embryo, often resulted in death losses of 50% or greater. Investigators using 20mg/embryo often do not mention mortality or whether dead embryos were analysed.

These results in the chick agree with investigations in other species. β -Aminopropionitrile decreased sulphate incorporation into chondroitin sulphates in rat cartilage *in vivo* and in calf cartilage slices *in vitro* (Karnovsky & Karnovsky, 1961).

The chondroitin 4-sulphate-protein linkage region is the neutral trisaccharide galactosylgalactosylxylose (Roden & Smith, 1966) and could be a site for control of glycosaminoglycan biosynthesis. The biosynthesis of this linkage region is dependent upon UDP-glycosyltransferases (Robinson *et al.*, 1966). The activities of both UDP-xylosyl- and UDP-galactosyltransferases were depressed by the nitrile *in vivo* (Table 2). The UDP-glycosyltransferase reactions are two-substrate reactions and one substrate is the endogenous acceptor protein present in the homogenate. The biosynthesis of the chondroitin sulphate complex requires protein synthesis (Telser *et al.*, 1965); therefore, decreased acceptor-protein synthesis could account for the observed effects of β -aminopropionitrile on transferase activities.

In any event the failure to demonstrate significant inhibition of these transferases by β -aminopropionitrile *in vitro* argues against any direct effect of the nitrile on these enzymes *in vivo*. The results *in vivo* then, in view of other information, are best interpreted as reflecting a decrease in protein synthesis either of enzyme protein or acceptor protein or both.

β -Aminopropionitrile clearly inhibits growth between day 7 and day 10 of embryonic development. With 9mg of nitrile given on day 7, 10-day embryos weigh about one-half as much as controls. Further,

injection of the nitrile inhibits O_2 consumption by embryo homogenates. A depression of O_2 consumption by β -aminopropionitrile has been reported in intact chick embryos (Clemmons, 1966) and in liver and cartilage slices (Juva *et al.*, 1959); these results support the contention that a block in oxidative metabolism is present. An additional finding *in vivo* is that embryo extracts are almost devoid of esterified *N*-acetylhexosamines (presumably UDP-*N*-acetylhexosamines). The common denominator of these findings is ATP; this is required for growth, protein synthesis, UDP-sugar formation and glycan synthesis. In view of the greatly impaired respiration by homogenates from nitrile-treated embryos one can reasonably expect any process that depends on oxidative metabolism to be impaired.

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