

Quantitation of glucosamine and galactosamine in limited hydrolysates of cartilage<sup>12</sup> indicate that the glycosaminoglycans present in annular cartilage contain mainly N-acetyl galactosamine and comprise less than 5% of the dry weight of the cartilage.

Samples of untreated cartilage as well as cartilage that had been treated with cyanogen bromide or hot alkali were prepared for electron microscopy (fig.). The matrix of untreated cartilage consisted of a dense network of branched fibrils and matrix granules (fig., a). The matrix fibrils showed no cross-banded periodicity and had a diameter of 150–400 Å. Matrix granules were spherical to polygonal shaped having a diameter of 100–400 Å. After treatment with either cyanogen bromide (fig., b) or hot alkali (fig., c), the fibrillar nature of the matrix was retained, though the diameter of the fibrils was reduced and matrix granules removed. Extraction of lamprey cartilage with 4 M guanidine hydrochloride which removes most proteoglycans from mammalian cartilage<sup>13</sup> removed matrix granules; however the matrix fibrils were unchanged.

The fibrous nature of this cartilage protein and its insolubility both suggest that it may be regarded as a structural protein. However, its amino acid composition clearly distinguishes it from other previously identified structural proteins including elastin<sup>7</sup>, abductin<sup>14</sup>, collagen, resilin, elastoidin or silk fibroin<sup>15</sup>. Although the physical properties of the protein imply that it exists as a crosslinked polymer, such crosslinks do not seem to be of the types present in insoluble elastin.

Although collagen represents 40–80% of the dry weight of most vertebrate cartilages<sup>16</sup>, it clearly can be no more than a minor component of lamprey cartilage. Thus we have shown that lamprey cartilage is not a hyaline cartilage, nor does it contain any elastin. The present amino acid analysis and corresponding electron microscopic examination demonstrates that the cartilage of the adult lamprey is an unusual form of vertebrate connective tissue composed primarily of a previously unrecognized but major structural protein of unique composition which we have termed lamprin.

Despite the position of lampreys among the vertebrates, they are specialized in many ways<sup>17</sup> and this new type of connective tissue may not necessarily represent a primitive kind of cartilage but one which has developed through the 280 million years of the evolution of lampreys.

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## Plasma catecholamines in children

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**Summary.** Plasma catecholamine concentrations in 46 children of various ages were determined by a sensitive radioenzymatic assay. Noradrenaline levels were found to be in the same range as in adults, whereas adrenaline levels in a few of the children were abnormally high.

Little information is available concerning the physiology and pathophysiology of the sympatho-adrenal system in children. Recently, radioenzymatic methods have been introduced which allow to determine even in little children in small volumes of blood the plasma adrenaline (A) and noradrenaline (NA) concentrations<sup>1</sup>. The concentrations of A and NA in plasma are commonly accepted as the best and most reliable parameters of sympatho-adrenal activity.

**Subjects and methods.** 32 'healthy' children from the orthopedic clinic and 14 children from the cardiology clinic were investigated (see fig.). The 'healthy' children had been hospitalized for conservative treatment or surgical correction of club-foot, hip luxation or os tibiale externum. Before conservative or surgical treatment was started, a blood sample of 3 ml was drawn from the child's cubital

vein immediately after venipuncture. During this procedure all the children were lying in bed and their arm was fixed by an assistant. These 'healthy' children had received no medication, and the collection of blood for the catecholamine (CA) assay was carried out in all the children at the same time of day (8.30–10.30 h). The children from the cardiology clinic were hospitalized for cardiac catheterization. None of the children was in frank heart failure at the time of the study, although half of them were receiving digoxin. The blood sample of 3 ml was taken from the right heart during catheterization. These children were premedicated with atropine and sedated with diazepam and/or fentanyl. Concentrations of A and of NA in the blood sample were determined by a radioenzymatic assay<sup>1</sup>.

**Results and discussion.** As may be seen from the figure, the

most striking but misleading result of the study is the abnormally high plasma NA level observed in the healthy, unsedated, very young children (age 4 months to 2 years). An age dependency for the level of plasma NA in children could be assumed from this result.

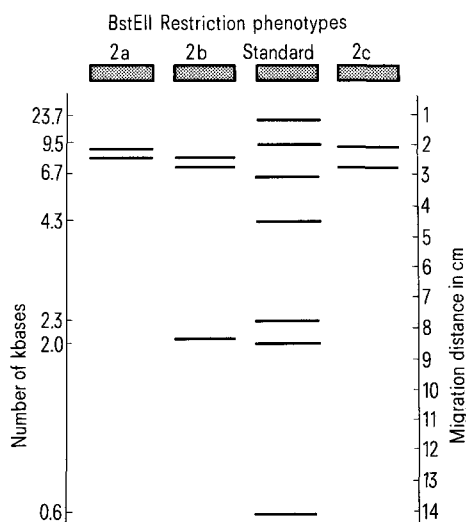
However, this assumption does not hold true, because in the control group of children who were under sedation, no such a difference in the NA levels between very young and older children was observed (fig.). Furthermore, the NA levels of the sedated very young and the sedated older children did not differ from the NA levels obtained from the other healthy, unsedated children above 2 years of age. Undoubtedly, the results from healthy, unmedicated children cannot be compared directly with results from children who were sedated and were under heart catheterization. However, if plasma NA concentration is really age dependent in children, then this must also hold true for the children under sedation during heart catheterization; this was not the case. So, except for the abnormally high plasma NA levels in the unsedated very young children, the normal non-basal plasma NA level in children was in the range of

0.40 ng/ml, without substantial variation between the different age groups (fig.). The plasma NA concentration is not different from the basal plasma levels commonly observed in adults<sup>2-4</sup>.

No differences in the plasma A concentrations were found, either between the young and the older children or between the sedated and unsedated children. Although in most of the children the A levels were as low as in adult subjects (range 0.04–0.14 ng/ml plasma), 4 of the 32 healthy unsedated children and 3 of the 14 sedated children had A levels which were abnormally high (0.41–0.49 ng/ml plasma) and in the same range as their NA concentration. Such abnormally high plasma A levels cannot be observed in adults under resting conditions<sup>5</sup>. An explanation for this finding in children cannot be given; a technical error is excluded.

The plasma CA concentrations given for healthy children in this study must not be taken as basal values. True basal plasma CA levels can be obtained only from unmedicated subjects under defined physical and psychological resting conditions; taking the blood sample for the assay without any pain to the subject by means of an indwelling catheter. In this respect our study is of great practical significance; showing that the child's level of agitation is highly influential in determining the actual plasma NA level in the very young. However, in children older than 2 years of age, the actual plasma NA concentration is obviously not substantially influenced by excitement, hence similar values were obtained in sedated and unsedated children.

Our results suggest that in children older than 2 years of age the plasma CA concentration measured in samples taken from the cubital vein under non-basal conditions is normally sufficiently accurate to exclude a severe pathological derailment of the sympatho-adrenal system. In contrast, in very young children, misleadingly high plasma NA levels must be expected if blood is taken without observing true resting conditions.



Plasma concentrations of noradrenaline and adrenaline (mean  $\pm$  SD) in children of various age: Comparison of the results from healthy unsedated children and sedated children.

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## Influence of fasting and of a high-protein diet on the activity of rat liver $\gamma$ -glutamyl transferase

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**Summary.** Fasting for 2 and 4 days progressively increases the activity of hepatic  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) in rat. A high-protein diet (with 42.6 energy percent of protein) for 45 and 90 days inhibits it. It seems that liver  $\gamma$ -GT is susceptible to nutritional influences.

The enzyme  $\gamma$ -glutamyl transferase ( $\gamma$ -GT, EC 2.3.2.2) attracts attention because of its presence at significantly increased levels in serum in a number of hepatobiliary disorders – cholestasis, hepatomas, alcoholic injuries, porphyria cutanea tarda, and the effects of drugs and chemicals<sup>1-4</sup>. Studies of possible nutritional effects on liver  $\gamma$ -GT are scarce<sup>5,6</sup>. We have measured the activity of hepatic  $\gamma$ -GT during fasting and during a more prolonged high-protein diet.

**Material and methods.** Female Wistar albino rats, weighing

150–180 g were used. In experiment I the animals were divided into the following groups; nonfasted controls; rats fasted 2 days; rats fasted 4 days; rats re-fed for 2 days after a 4-day fast; rats re-fed for 4 days after a 4-day fast. The animals were housed in individual wire-bottom cages and were allowed free access to a commercial pellet diet except during the fasting periods. No mortality was observed on the 2nd day of food deprivation, but 20% of the animals died on the 4th day of fasting.

Experiment II included 4 groups: rats fed for 45 days on a