The Mucopolysaccharides of 
_Nucleus Pulposus_

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From sulphate and monosaccharide analysis it has been concluded that the mucopolysaccharides of human _nucleus pulposus_ is chondroitinsulphuric acid and keratosulphate.

These two polysaccharides have now been isolated and identified. A dried preparation of _nucleus pulposus_ was heat-coagulated and digested with glycerol extracts of pancreas and of intestinal mucosa followed by precipitation with alcohol. The precipitate was extracted with phenol and the digestion and extraction procedure repeated once.

In this way a polysaccharide fraction with a ratio nitrogen/amino sugar of 1.5 was obtained. Glucosamine and galactosamine were both present in a ratio of 0.8.

The fractionation of the purified material was most easily accomplished by precipitation with ethanol on top of a cellulose column followed by elution with ethanol in decreasing concentrations.

Two peaks were obtained, when the effluent was analysed with Dische’s carbazol method. In the first peak appearing at about 35% ethanol the aminosugar component was made up to 90 to 95 per cent of glucosamine. The second peak appeared at an alcohol concentration of 10–15%, and 95 to 100% of the aminosugar was galactosamine. Galactose was found only in the first peak.

Details of the fractionation procedure together with analysis and properties of the fractions are given.


The Effect of pH on the Balance between Oxidation of Ascorbic Acid and Reduction of Dehydroascorbic Acid in Plant Tissue

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A rapid oxidation of ascorbic acid in raw cabbage leaves when sprinkled with diluted acetic acid has been demonstrated. We were able to show that this oxidation is brought about by the ascorbic acid oxidase of cabbage. The same effect of acetic acid is obtained in a large number of fruits and vegetables containing strongly active ascorbic acid oxidizing enzymes.

Further experiments have shown that in raw cabbage reduction of dehydroascorbic acid (DHA) by reduced glutathione under anaerobic conditions is much more sensitive to lowering of pH than aerobic oxidation of ascorbic acid (AA). Thus, the effect of acetic acid may be explained by a stronger inhibition of reduction of DHA in an acidified cell as compared to oxidation of AA.

2. Alm, F. Intern. Z. Vitaminforsch. 23 (1952) 459.
3. Alm, F. Ibid. 24 (1952) 81.

A New Abnormal Fe-Hemochromogen as a Cause of Hereditary Cyanosis

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The authors have investigated the blood from a family with hereditary non cardiac cyanosis affecting 5 of 7 members representing three generations.

The maximal oxygen combining power was 20% less than for a normal blood with the same iron content. Absorption spectral curves of oxygenated blood as compared with oxyhemoglobin from normal blood with the same iron content showed neither differences within the ultraviolet range (240 mµ-400 mµ) nor within the infrared (750 mµ-1000 mµ). In the visible range there was found increased absorption from 480 mµ-520 mµ and between 590 mµ-670 mµ with a maximum at 600, and decreased absorption between 530 mµ-555 mµ indicating the presence of an abnormal hemochromogen. The non-identity with methemoglobin (MHB) and sulfhemoglobin was evident from different absorption spectral curves and further differing from MHB by the absence of reaction with cyanide and fluoride.
as well as from failing response in vivo to methylene blue or ascorbic acid (reduction of MHB to ferrohemoglobin). The abnormal hemochromogen could not be transferred to MHB by ferricyanid, nor could it be reduced by hydrosulfite or transferred to COHb, as judged from absorption spectral curves, where-as it could be transformed quantitatively to acid hematin. The peroxidase activity of the blood was the same as for normal blood with the same iron content. No difference from normal hemoglobin in electrophoretic mobility (paper electrophoresis) was found at pH 8.6.

It is concluded that the abnormal hemochromogen assumingly is a Fe-chromogen with peroxidase activity but not able to combine with oxygen and responsible for a new form of hereditary cyanosis. The hemochromogen is called Norin-hemochromogen referring to the name of the cyanotic family.

Cholyl-CoA as an Intermediate in Taurocholic Acid Formation by Rat Liver Microsomes

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We have previously shown that both the microsomal fraction and the particle-free supernatant of rat liver homogenates are required for the conjugation of cholic acid with taurine 1-4.

The mechanism of this conjugation has been subjected to further studies.

Rat liver microsomes were isolated as follows: The liver was homogenized in 4 parts 10 % sucrose. Nuclei and mitochondria were sedimented at 16 000 g for 15 min, the microsomes at 25 000 g for 110 min. The fraction was washed once.

The taurocholic acid formation was followed by means of 3H-labelled taurine as previously described 1. It appears from Table 1 that the particle free supernatant could be substituted by CoA and ATP. The conjugation was also stimulated by Mg++ (though not in the published experiment) and fluoride. Cysteine or glutathion was required when a crude CoA was used, but when a reduced, 70 % pure CoA was used, the effect of cysteine or glutathion was slightly inhibiting.

Table 1. Complete system: Phosphate buffer pH 7.4 0.022 M; KF 0.25 M; Cysteine 0.007 M; CoA 0.001 M; ATP 0.007 M; Mg++ 0.001 M; Sucrose 0.035 M; Microsomes from approximately 500 mg liver per vessel.

<table>
<thead>
<tr>
<th>Experiment I</th>
<th>Experiment II</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 µmoles taurine/vessel</td>
<td>1 µmoles cholic acid/vessel</td>
</tr>
<tr>
<td>2 µmoles cholic acid/vessel</td>
<td>Total volume 1.5 ml/vessel</td>
</tr>
<tr>
<td>Total volume 1.5 ml/vessel</td>
<td></td>
</tr>
</tbody>
</table>

All solutions were adjusted to pH 7.4. Gas phase air, Incubation time 120 min, temp. 37°C.

<table>
<thead>
<tr>
<th>Factor excluded from the complete system</th>
<th>Experiment I</th>
<th>Experiment II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Taurocholate formed</td>
<td>Hydroxamic acid formed</td>
</tr>
<tr>
<td></td>
<td>µmoles/vessel</td>
<td>µmoles/vessel</td>
</tr>
<tr>
<td>None</td>
<td>0.31</td>
<td>0.27</td>
</tr>
<tr>
<td>Cholic Acid</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>KF</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>ATP</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CoA</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.34</td>
<td>0.35</td>
</tr>
<tr>
<td>Mg++</td>
<td>0.41</td>
<td>0.36</td>
</tr>
<tr>
<td>Hydr.-oxylamine</td>
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</tr>
</tbody>
</table>

These findings make it probable that cholic acid is conjugated with taurine with cholyl-CoA as an intermediate. This was further supported by the formation of cholic hydroxamic acid when hydroxylamine was added to the incubation mixture instead of taurine.

The cholic hydroxamic acid formed was isolated by a modification of Eriksson and Sjövall's 5 quantitative paper chromatography for conjugated bile acids. A mixture of 1 part n-butanol and 2 parts petroleum spirit (b.p. above 120°C) was used as a moving phase. The hydroxamic acids spots were eluted with ethanol, the eluate was blown to dryness and the residue dissolved in conc. H2SO4. After 1 hour the density at 359 µ was measured.

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