The various sugar phosphates in the precipitate could not be separated by preparative paper chromatography or by paper electrophoresis. The mixture was, therefore, dephosphorylated by incubation in 0.05 *M* acetate buffer at pH 5.2 for 1 h at 30° C with a partially purified acid phosphatase preparation. Protein was precipitated with trichloroacetic acid and the resultant mixture was put through a mixed bed ion exchange resin column (IR120 (H^+) and IRA400). The free sugars were then separated by paper chromatography in phenol-water (4:1)[13] on long papers (36 inches). The ketopentose areas were made visible by applying a new combination spray. The TCA-orcinol spray of KLEVSTRAND AND NORDAL[14] was applied first (with subsequent heating) followed by aniline phthalate[15] overspray (without heating). This procedure produced a purple color with D-xylulose and a pink color with D-ribulose but gave no color with aldopentoses.

D-Xylulose was identified as one of the major components of the mixture of sugars by the specific color, by its position on paper and by conversion to its *p*-bromophenylhydrazone. The eluted material was, however, contaminated with traces of D-ribose which occupied the same position on the papers but gave the typical aldopentose reaction when sprayed with aniline phthalate alone. The D-ribose in the eluate was converted by bromine oxidation to ribonic acid which was removed from solution by passage through a mixed bed ion exchange resin. The ketopentose remaining in solution was characterized by preparation of its *p*-bromophenylhydrazone (mp: 128–9° C) by a modification of the original method of SCHMIDT AND TREIBER[16]. A mixture of this derivative and an authentic sample of D-xylulose-*p*-bromophenylhydrazone (m.p. 128.5–129.5° C) melted at 128–129.5° C. The presence of D-xylulose phosphate in the sugar phosphate fraction was thus established. Although the position of the phosphate group in the molecule could not be determined, the acid stability of all the phosphorylation products[1] suggests that the compound in question is D-xylulose-5-phosphate.

No D-xylose was detected after dephosphorylation of the sugar phosphates. Moreover, D-xylose-5-phosphate** was not further metabolized by the enzyme system. This result is in agreement with earlier findings[17] and with the suggestion that isomerization precedes phosphorylation[6].

The experimental data given in this communication establish the initial sequence in the utilization of D-xylose by extracts of *P. hydrophila* as:

$$\text{D-xylose} \rightleftharpoons \text{D-xylulose} \longrightarrow \text{D-xylulose-(5)-phosphate.}$$

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Some properties of the casein of mouse milk (RIII)*

A protein, considered to be casein on the basis of its phosphorus content, has been prepared from the milk of mice of the RIII strain (carriers of the mammary tumor agent). Its concentration is approximately 2.8%. Some of its properties are described below.

The manner of collecting the milk and the precautions taken to avoid changes during treatment

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have been mentioned in describing an alcohol-soluble protein from the same source[1]. Following removal of lipides and cellular elements from the milk by centrifuging at 1000 *g* the casein was sedimented at 14,000 *g*, dissolved in water and again sedimented at this centrifugal force. From its dialyzed, milk-white, water solution the protein was precipitated by adding several volumes of citrate buffer, pH 3.1, washed repeatedly with water, dissolved in a minimal quantity of NaOH, or phosphate buffer or barbiturate buffer, as required. The casein was no longer sedimentable at 14,000 *g* at this stage and its solutions were clear and completely free of any milky appearance.

A water solution formed no coagulum on boiling. The casein was precipitated by the usual protein-precipitating agents but not by 4 volumes of absolute ethanol, although prior addition of a little $CaCl_2$ or sodium phosphate resulted in abundant precipitation by the ethanol. Results were positive when color tests were applied to the casein solution for the presence of tyrosine and tryptophan. A paper chromatogram of 0.42 mg, following hydrolysis with HCl, contained spots corresponding to leucine, glutamic acid, valine, alanine, lysine, glycine, serine, tyrosine, proline, arginine(?), methionine and aspartic acid. Absorption of light in the ultraviolet region was characteristic of proteins in general, the maximum being at 278 mμ in *N*/20 HCl and shifting to 290 mμ in *N*/20 NaOH. A sample of casein prepared by precipitating with HCl, instead of citrate as described above, and well washed with water, contained 1.2% phosphorus and 15.34% nitrogen (micro-Dumas), uncorrected for ash content.

Electrophoretic examination. This was done at 0°–0.5° on dialyzed solutions in a Tiselius apparatus. The mobilities (10^{-5} cm^2 volt^{-1} sec^{-1}) were −5.4 and −7.3, respectively, at pH 5.80 and 6.92 in phosphate buffer, calculated from the data for the descending limb of the cell. When the concentration, which was *ca.* 1.9% in the latter experiment, was reduced to *ca.* 0.6% the mobility was −6.3. In barbiturate buffer at pH 8.58 it was −6.2 and −7.0 for 0.4% and 0.9% solutions respectively*. Mobilities were 12 to 18% greater in the ascending limb.

A small peak, representing about 8% of the total area, is present in the pattern obtained at pH 8.58; it is smaller at pH 6.92 and barely apparent at pH 5.80. It is so close to the peak for casein that it may be that it represents a partially dephosphorylated casein (with a slightly smaller net charge) present in the freshly collected milk, or formed subsequently. Partly dephosphorylated casein has been identified following treatment of α-casein of cow's milk with either prostatic or intestinal phosphatase[4].

The isoelectric point of the casein could not be determined electrophoretically owing to its very limited solubility below pH 5. When 1 ml of a solution previously dialyzed against water was added to 5 ml quantities of 0.1 *M* acetate buffer with graded pH's precipitation was maximal at pH 4.3 ± 0.1. Of the 10.20 mg casein added 0.12 mg remained in solution.

Viscosity. A sharp rise in viscosity of the casein solutions occurred with increasing concentration. Thus, 0.4%, 0.8%, 1.2%, 1.66% and 2.5% solutions had, at 0°, viscosities relative to the buffer solvent, of 1.021, 1.109, 1.249, 1.394 and 1.656 as determined with the Ostwald viscosimeter.

*Ultracentrifugal examination***. When an 0.8% solution of casein in 0.1 *M* NaCl was centrifuged at 60,000 rpm, equivalent to 250,000 *g*, the value of $s_{20,w}$ was 27 *S*.

Diffusion. The diffusion constant D_{20}, measured at 0°–0.5° in a Tiselius cell on a 1.2% casein solution in phosphate buffer, pH 6.92 and containing 0.05 *M* NaCl (total μ = 0.1) was $2.34 \cdot 10^{-7}$ cm^2/sec. A second determination (0.6% solution) gave the value $2.31 \cdot 10^{-7}$ cm^2/sec. Dissociation into sub-units did not, therefore, occur on two-fold dilution in this concentration range. The maximal ordinate-area method was used in calculating D_{20}.

Calculation of the molecular weight of the mouse milk casein by means of the formula $M = RTs/D(1-V\varrho)$ yields a value of 1,130,000, if the partial specific volume is assumed to be 0.75. Since it was sedimentable at 14,000 *g* before, but not after, isoelectric precipitation, casein as it exists in mouse milk (calcium phosphate complex) must have an even greater molecular weight. Both as the calcium phosphate complex and as the sodium salt the large molecules may represent associations of submolecules. The frictional coefficient f/f_0 is 1.38 when computed from the same data, indicating that the axial ratio is between 7 and 8.

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* DAVIS AND COHN[2] found that mobilities increased with increasing concentrations of hemoglobin. LONGSWORTH AND MACINNES[3] observed the same for ovalbumin and indicated the causes for this and for the difference in mobility values for ascending and descending boundaries.

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