LIV. PHOSPHORUS COMPOUNDS OF MILK.

IV. PRESENCE OF ADENINE NUCLEOTIDE IN MILK.

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It has been shown by one of us [Kay, 1925] that there are present in protein-free filtrates from goat's, cow's or human milk, acid-soluble phosphoric esters which are readily hydrolysable by dilute acids and by extracts of certain tissues, and are readily diffusible through collodion membranes.

We have now separated one of these compounds which appears to be composed of adenine and a pentose in combination with phosphoric acid. It is present in goat's milk, but only in very small quantities. So far, we have not been able to demonstrate its presence in cow's or in human milk.

Jackson [1923, 1924] brought forward evidence to show that adenine nucleotide was present in blood, but he was not able to obtain analytical figures indicating purity, and believed that his substance was contaminated with an approximately equal quantity of a pyrimidine nucleotide. Hoffman [1925] has isolated crystalline adenine nucleotide from pig's blood. We have ourselves obtained a substance from goat's and from pig's blood which gives reactions for adenine, pentose and phosphoric acid combined together. In milk, however, the similar compound is present in much smaller quantities than in pig's or in human blood (accepting Jackson's figure of 15–25 mg. nucleotide per 100 cc. for the latter). The highest figure yet found for goat's milk is 3.3 mg. nucleotide, corresponding with about 0.3 mg. phosphorus per 100 cc. milk. The average organic acid-soluble phosphorus of goat's milk is some 13 mg. per 100 cc. The nucleotide that we have found accounts therefore for only a small fraction of the ester phosphorus. It is in fact so small in quantity that it may be only an adventitious milk constituent, derived from cellular breakdown in the mammary gland.

Method of separation¹.

Goat's milk in 300 cc. lots was diluted to 500 cc. with water and shaken thoroughly with excess of picric acid paste. After standing 15 minutes the mixture was filtered, and the combined filtrates from 10 l. of milk evaporated *in vacuo* to about 2½ l. A crop of potassium picrate separated on cooling. The filtrate was neutralised to Congo red with 10 % NaOH, and filtered. 450 cc.

¹ Based on Jackson's method, after several unsuccessful attempts to devise a method giving a better yield.

of 20 % uranyl nitrate were added, and the whole was kept overnight. The precipitate was filtered off and washed twice with water by grinding in a mortar. It was suspended in water, and solution effected by adding 2*N* H₂SO₄. The filtered solution was treated with 10 g. HgSO₄ dissolved in 2*N* H₂SO₄, and 10 % NaOH added until a copious precipitate formed. Fractionation at this stage did not appear to be of value. The mercury precipitate was decomposed with ammonium sulphide, excess of the latter being boiled off. A few cc. of magnesia mixture were added to precipitate free phosphate and flocculate the colloidal sulphur which was usually present. The filtrate was evaporated on the water-bath to 50 cc., and a solution of 3 g. AgNO₃ added. The nucleotide came down in the silver precipitate, which was decomposed with H₂S and the filtrate evaporated to about 5 cc. *in vacuo* at 35°. 10 volumes of alcohol were added, the precipitate was filtered off and dried. It gave P = 11.32 %, N = 14.2 % in one case. The substance was dissolved in water, reprecipitated in presence of dilute acetic acid as the lead salt, decomposed with H₂S, again precipitated with alcohol and dried. Two different specimens gave P = 11.3 %, 11.16 %, N = 16.42 %, 15.38 % (calculated for adenine nucleotide, P = 8.93 %, N = 20.1 %). At this stage the amount of substance present was of the order of 0.1 g., so that further purification was difficult. Fractional precipitation from aqueous solution with alcohol was of no avail. Specimens prepared by slightly different methods from goat's milk had at this stage similar analytical figures, but showed too high a phosphorus and too low a nitrogen content for pure adenine nucleotide. After four attempts (employing the above method or modifications of it), in which 20 gallons of goat's milk were used in all, we did not succeed in obtaining a product which gave analyses agreeing with adenine nucleotide. The small yield of material precluded purification by crystallisation of the brucine salt.

The dry white powder thus obtained is readily soluble in water and gives the following reactions. (a) It is acid to methyl red. (b) It contains no free phosphate. (c) On boiling with strong HCl it gives a typical pentose reaction with orcinol, phloroglucinol or with Bial's reagent. The pigments produced with these reagents are soluble in amyl alcohol, the extracts showing absorption bands on the green or the red side of the sodium *D* line exactly corresponding with those given by an authentic specimen of arabinose, similarly treated. (d) The aqueous solution gives no precipitate with ammoniacal silver nitrate, but after heating for 2½ hours with *N*/2 sulphuric acid the reaction mixture gives, with excess of ammoniacal silver nitrate, a precipitate, from which, after decomposing with HCl and removing the silver chloride, a crystalline picrate may be obtained. After two recrystallisations from water, it has m.p. 292° and N = 31.2 % (calculated for adenine picrate, N = 30.8 %). A mixed melting point with an authentic specimen of adenine picrate is unchanged. The crystalline form of the two is identical. (e) In 2½ hours, *N*/2 sulphuric acid, which is stated to decompose adenine nucleotide completely in this time, removes about half of the organic phosphorus. (f) Kidney

phosphatase at 38° completely hydrolyses the ester phosphorus to inorganic phosphate in 4 hours. (g) There is no reduction on boiling with Fehling's solution.

It seems clear that adenine nucleotide is present, either mixed or in loose combination with an impurity containing more phosphorus and less or no nitrogen. The contaminating substance may be diphosphoglyceric acid ($P = 23.4\%$). It is probably not a pyrimidine nucleotide, as the percentage of phosphorus found is higher than that of any known nucleotide. For comparison, the nucleotide from 2 l. of pig's blood was prepared in a similar way. A substance was obtained containing $P = 11.3\%$, $N = 16.0\%$, and identical in all the above reactions with the milk compound.

SUMMARY.

Adenine nucleotide, associated with some other phosphoric ester with similar precipitation reactions and solubilities, has been shown to be present in very small quantities in goat's milk. Its concentration is of the order of 3 mg. nucleotide per 100 cc. milk.

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