point, the following experiment has been carried out:

A calcium phosphate precipitate was prepared from Sørensen's secondary phosphate and a slight excess of calcium nitrate. After standing overnight the supernatant was decanted and an excess of cobalt nitrate added. The mixture was stirred over night, the supernatant was decanted, the precipitate resuspended in water and divided into two parts.

One part was treated with sodium sulfide and the resulting black precipitate filtered, washed and dried. The other part was washed and dried and then suspended in a 5 % agar solution. After gelation a cube of 2 cm side length was cut from the agar and immersed in sodium sulfide solution. Within 30 minutes the black coloration of cobalt sulfide had extended to the core of the cube. Accordingly, the main portion of the agar suspension was sliced in 2—3 mm thick discs and treated with sodium sulfide for 3—4 hours. Then the blackened precipitate was recovered by melting, diluting and decanting. Both preparations were then analyzed with the following result (values in % of water-free precipitate).

	Water	Agar
Sulfide-S	3.5	2.4
Co	22.0	26.5
PO_4	21.5	18.4

Besides, there was a very heavy qualitative reaction for SO_4^{--} and a somewhat slighter one for Ca^{++}. Calculated for CoS: Co 64.8 %, S 35.2 %, for Co_2S_3 : Co 55.1 %, S 44.9 %.

From the above experiments we have to conclude that the conversion of calcium phosphate into cobalt sulfide is by no means quantitative. Moreover, a substantial part of the cobalt sulfide, even if formed originally, seems to be oxidized to sulfate. Since this is the case even in model experiments it is to be assumed that in tissues the process of conversion will be still more complicated. As long as cobalt sulfide is formed at all, the value of the reaction as a qualitative method of phosphatase detection will scarcely be influenced by the results reported here; but in all cases where the intensity of blackening is taken as a quantitative indicator of the amount of phosphatase present, the greatest caution seems to be necessary. Numerous such attempts have been reported in the literature.

The only case that we know of in which the formation of sulfide has been evaluated quantitatively is an investigation by Doyle [1], where the sulfur is extracted and incorporated into methylene blue which is measured colorimetrically. The detailed communication about this method has not yet appeared, but Dr. Doyle has informed us that in this case, too, the conversion into sulfide has been found to be incomplete.

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Note on the Crystallisation of Chondrosamine

SVEN GARDELL

Chemistry Department II, Karolinska Institutet, Stockholm, Sweden

In working with mucopolysaccharides it is often necessary to use chondrosamine (galactosamine) as a reference substance. However this substance is very difficult to obtain pure in any reasonable yield. The best method is that originally described by Levene [1], in which the lead salt

or the barium salt of chondroitinsulfuric acid is hydrolysed with strong hydrochloric acid in the presence of stannous chloride. After removal of the heavy metals with H_2S the filtrate is evaporated to a syrup. This is taken up either in ethyl alcohol from which the chondrosamine hydrochloride crystallises on the addition of ether or in methyl alcohol to which aceton is cautiously added. Working with this method we very often found that a sticky mass appeared instead of crystals when ether or aceton was added or, when the mass did not appear, the yield was very poor. The frequent appearence of this sticky mass was sometimes due to incomplete hydrolysis. It was however found that when the aceton was sufficiently cooled satisfactory crystallisation was sometimes obtained in batches where a sticky mass had at first appeared. Improvements in the technique therefore seemed necessary. Several modifications having been tried we found the following technique the most suitable.

50 g chondroitinsulfuric acid, prepared from bovine tracheal cartilage according to the method of Strandberg [2], were dissolved in about 50 ml of water. Hydrochloric acid was added until the solution turned acid to Congo. The chondroitinsulfuric acid was precipitated with 5 volumes of alcohol, the precipitate washed with alcohol, dissolved in 500 ml of water and the solution neutralized with $Ba(OH)_2$ to faintly alkaline reaction. The solution was clarified by centrifugation and made neutral or faintly acid (pH 6.5) with dilute hydrochloric acid and the barium salt of the chondroitinsulfuric acid precipitated with 2 volumes of alcohol.

The barium salt from 50 g of chondroitinsulfuric acid thus obtained was hydrolysed over free flame under reflux for 7—8 hours in 500 ml of 20 % hydrochloric acid in the presence of 10 g stannous chloride. After a few minutes the solution turned brown and at the end of the hydrolysis the reaction mixture was black. It was poured into 2 liters of hot water and treated with H_2S. The solution was filtered and the clear faintly yellow solution aerated to remove the excess of H_2S. The filtrate was then evaporated under reduced pressure to dryness. The residue was then extracted with about 100 ml of methyl alcohol to which 5 ml of 1—N hydrochloric acid had been added. The insoluble part, consisting to 85 % of ash, was removed by filtration. The filtrate was placed in the icebox at —10° C for one hour. Cold (—5° to —10° C) aceton was then added very cautiously through a pipett under vigorous mixing until a faint opalescence appeared. The walls of the vessel were scratched and the solution left in the icebox over night. The following day the crystals formed were scraped down from the walls, aceton added again and the solution left in the icebox. This treatment was continued for 4—5 days until no more crystals appeared. The crystals formed were removed by filtration, washed first with methyl alcohol containing 10 % of aceton and 1 % normal HCl, and finally with pure aceton, and then dried over P_2O_5. Yield 9.8 g of chondrosamine hydrochloride (52 % of theoretical). This preparation is sufficiently pure for most purposes. It analysed as follows:

	Found	Theoretical
Moisture	0.42 %	—
Ash	< 0.05 »	—
Nitrogen:		
(Micro Kjeldahl)	6.51 »	6.5 %
Chlorine	16.1 »	16.5 »
Melting point:	179° C	182° C (Levene)

a_D^{20} (2 % solution) initial + 62°, final 92.5° (Levene + 96.4°).

After evaporation of the mother liquor to dryness and treatment as above another lot of 2 g of the hydrochloride was obtained. Total yield 63.7 %.

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