LXVI. NOTE ON THE PREPARATION OF PURINES AND PYRIMIDINES FROM NUCLEIC ACID

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THE present methods [Jones, 1920; Levene, 1922] for the preparation of purines and pyrimidines from yeast nucleic acid can be shortened considerably. Advantage is taken of the difference in solubilities of the hydrochlorides of guanine and adenine in one instance, and of cytosine hydrochloride and uracil in another. Separation and purification can thereby be effected simply without the intermediate formation of Cu or Ag salts or picrate. Moreover, all four substances can be obtained from the same sample of nucleic acid. The following is the method adopted.

Purines

100 g. nucleic acid are suspended in 500 ml. of 95% methyl alcohol and the suspension is saturated with a vigorous stream of HCl under reflux. The saturation and hydrolysis require about 3 hr., during which time the solution is kept hot by the rapid passage of HCl. The solution becomes dark, and the original nucleic acid in suspension is replaced by a precipitate of guanine and adenine hydrochlorides. The reaction mixture is set aside overnight.

The precipitate is collected, washed with methyl alcohol and dried. The conditions of hydrolysis are such that the precipitate at this stage weighs 28–30 g. If double the volume of alcohol is used (Levene's conditions) and hydrolysis is allowed to proceed for 2 hr. a much larger precipitate is obtained. From the methyl alcoholic filtrate a further small precipitate is obtained on concentration to 250 ml. The final methyl alcoholic filtrate and washings are reserved for pyrimidine recovery.

The combined precipitate is now dissolved in 350 ml. hot water, boiled with charcoal and filtered hot. No acid is introduced at this stage so that maximum decoloration may take place. Sufficient concentrated HCl is added to the decolorized aqueous filtrate to make it 2*N* with respect to HCl, any precipitate is redissolved by heat and the guanine hydrochloride is allowed to crystallize slowly. It is very insoluble and precipitates almost quantitatively. A few crystals of adenine hydrochloride, appearing as sago-like clusters, may also appear at this stage, but remain in the mother-liquor on recrystallization.

The guanine hydrochloride crystals are collected; if they are washed with dilute HCl and air-dried, the dihydrate is obtained, but if washed with alcohol and oven-dried, the crystals lose their lustre and are converted into anhydrous guanine hydrochloride. Pure guanine hydrochloride is obtained by recrystallizing from 200 ml. 2*N* HCl. Yield 9.8 g. (oven-dried).

The filtrates from guanine are now combined and filtered from any precipitate that may have formed on standing. The solution is reduced *in vacuo* to a small volume until crystallization begins. Adenine hydrochloride precipitates quickly. It is purified by recrystallizing from a small amount of water. Yield 8 g.

Pyrimidines

Although synthetic methods of obtaining pyrimidines are simpler, their preparation as by-products of nucleic acid hydrolysis deserves consideration.

From the fraction reserved above for pyrimidine preparation, alcohol is removed by distillation. The dark, syrupy residue is taken up in 400 ml. 20 % HCl and autoclaved at 175° for 2 hr.

The carbonized mass obtained on autoclaving is leached with hot water. The solution is then evaporated to dryness *in vacuo* to remove excess HCl and the residue taken up in 500 ml. water. The amount of H_3PO_4 present is roughly calculated from the quantity of nucleic acid used, and $\text{Ba}(\text{OH})_2$ is added in excess. NH_3 is removed by distillation *in vacuo*. $\text{Ba}_3(\text{PO}_4)_2$ is then removed by centrifuging and the excess Ba is removed exactly from the supernatant fluid with H_2SO_4 . The resulting solution is concentrated *in vacuo* until crystallization begins. It is then boiled with charcoal, filtered and set aside. Crusty, granular crystals of uracil are deposited on the sides of the flask. A second crop is obtained by further concentrating the filtrate. Uracil is recrystallized in the usual way. Yield about 3 g.

The filtrates from the above are combined, reduced to 5–10 ml. and several volumes of absolute ethyl alcohol are added. Cytosine hydrochloride, which is very soluble in water but insoluble in alcohol, is precipitated readily. It is recrystallized in the same way from water and alcohol; yield about 2 g.

The yields of uracil and cytosine are only roughly quantitative and are considered low.

The method outlined has been used successfully in preparing large amounts of guanine, and recovering adenine, uracil and cytosine as valuable by-products. It is simpler and shorter than methods previously employed.

REFERENCES

- Jones (1920). *Nucleic Acids*, pp. 106–7. (Longmans, Green and Co., London.)
Levene (1922). *J. biol. Chem.* **53**, 441.