be considered an obligate autotroph, absolutely requiring both light and carbon dioxide for growth. This is analogous to the situation in *Thiobacillus thiooxidans* (*9*), where sulfur and carbon dioxide are required for growth and can be replaced by no other energy or carbon source, respectively.

Possibly some simple compound that we have overlooked will suffice to support growth in darkness. It may be, however, that for the growth of some organisms, such as *Chl. Moewusii* and *Th. thiooxidans*, one type of energy source is not replaceable by another. Herein lies the problem of obligate autotrophy.

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A Coenzyme of Spleen β-Glucuronidase[1]

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The preparation of an electrophoretically homogeneous β-glucuronidase from calf spleen has been described recently (*1*). The activation of β-glucuronidase by nucleic acids (*2*) and the existence of naturally-occurring inhibitors of β-glucuronidase (*3*) have been pointed out.

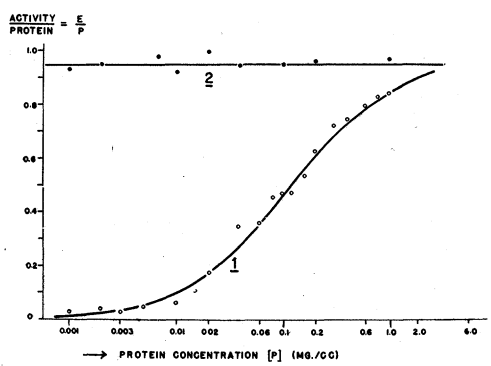

FIG. 1.

It has now been found that the activity of pure spleen β-glucuronidase is not proportional to the enzyme concentration, in contrast to the behavior of less pure preparations. The ratio of enzyme activity to protein concentration (E/P) drops greatly with dilution. Our experimental activity values (circles, Fig. 1) with increasing dilution coincide with a theoretical curve (Curve 1, Fig. 1) obtained from the usual dissociation expression:

$$\frac{(P-E)(C-E)}{(E)} = K, \qquad (1)$$

where P = total protein, C = total coenzyme, E = active enzyme, $\frac{P}{C}$ = constant, and K, a constant.

However, the addition of a boiled and filtered solution of the pure enzyme to dilute β-glucuronidase produces a strong activation (Table 1). This behavior is consistent with the view that spleen β-glucuronidase contains a thermostable coenzyme that dissociates on dilution. Furthermore, in the presence of 0.3% desoxyribonucleate the activity of pure β-glucuronidase is proportional to the protein concentration; i.e., E/P becomes independent of the protein concentration (black dots, Fig. 1). The distance on the ordinate between open circles and black dots indicates the activation by DNA for each protein concentration. This is very considerable at low protein concentrations.

TABLE 1

Protein concentration (mg/ml)	$\frac{\text{Activity}}{\text{Protein}} = \frac{E^*}{P}$	$\frac{E}{P}$ in the presence of boiled enzyme†	Activation by boiled enzyme
0.08	0.46	...	.....
.025	.23	0.41	1.8 fold
.02	.17	.44	2.6 "
.015	.11	.37	3.4 "
0.01	0.064	0.38	6 "

* The ratio E/P is given the value 1 when 0.165 mg phenolphthalein glucuronide is hydrolyzed in 1 min at 37° C by 1 mg protein in a digest of 1 ml.

† The concentration in the digest of the thermostable matter added with the boiled and filtered enzyme corresponds to 0.08 mg protein/ml before boiling.

When the activity of a dilute solution of pure glucuronidase (0.01 mg protein per ml) is determined in the presence of increasing amounts of desoxyribonucleic acid (DNA), and when the ratio of the enzyme activity to the protein concentration is plotted against the log of the DNA concentration, another S-shape curve is obtained. This experimental curve is in accordance with equation (1) when C becomes $(C+N)$, and N = concentration of DNA. This generalized equation also explains why E/P becomes independent of the protein concentration in the presence of a large excess of DNA (Curve 2, Fig. 1).

Accordingly, the addition of DNA in this system has the same effect as if more of the coenzyme were being added. However, we have no evidence that DNA is indeed the coenzyme. Yeast ribonucleic acid (RNA) gives a similar but somewhat smaller effect. For both DNA and RNA the activating effect is independent of their degree of polymerization.

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C^{14} Labeled 4(5)-Amino-5(4)-Imidazolecarboxamide in the Biosynthesis of Purines[1]

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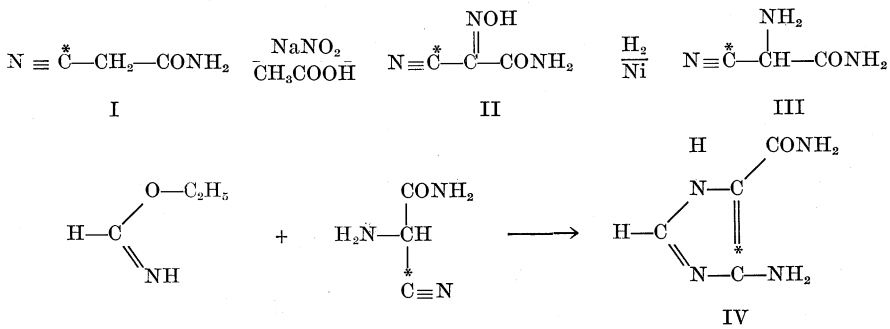
In a recent report from this laboratory (*1*), it has been shown that pigeon liver homogenates can convert 4(5)-amino-5(4)-imidazolecarboxamide (IV) to hypoxanthine, whereas the intact pigeon transforms the substance into uric acid. As part of a general program to study the biosynthesis of purines from labeled precursors, we have developed a convenient synthesis of 4(5)-amino-5(4)-imidazolecarboxamide containing C^{14} in the 4(5) position. Recently two new syntheses of this compound (nonlabeled) have been reported (*2, 3*).

Sodium cyanide (6 g) containing 0.6 mc of radioactivity as C^{14} and chloroacetic acid (12.7 g) were converted to cyanacetamide (I) by a modification of procedures described in *Organic Syntheses* (*4, 5*). The resulting labeled cyanacetamide was diluted with nonlabeled cyanacetamide (total, 16.75 g) and subsequently nitrosated with sodium nitrite in dilute acetic acid at 10°–15° to give labeled nitrosocyanacetamide (II) (17.4 g). The nitrosocyanacetamide was reduced with hydrogen and Raney nickel catalyst in methanol solution to the corresponding unstable aminocyanacetamide (III), which was used immediately in the next step without isolation. The addition of ethyl formimino ether in 20% excess to the cold methanol solution of aminocyanacetamide gave the desired 4(5)-amino-5(4)-imidazolecarboxamide (IV) which was isolated and purified as the picrate (9.5 g pure); mp, 239°–240° C decomposition (cor.) when inserted in a bath preheated to 227°.



Analysis calculated for $C_{10}H_9O_8N_7$: C, 33.81; H, 2.56; N, 27.58. Found: C, 34.00; H, 2.51; N, 27.68.[2]

The picrate was conveniently converted to the hydrochloride by suspending it in dry acetone and rapidly saturating with dry HCl while maintaining the temperature constant; mp, 262.5°–263.5° decomposition (cor.) when inserted in a bath preheated to 245°.

Analysis calculated for $C_4H_7ON_4Cl$: C, 29.53; H, 4.34; N, 34.46; Cl, 21.83. Found: C, 29.78; H, 4.39; N. 34.30; Cl, 21.78.[2]

The picrate and hydrochloride respectively were found to be identical with samples of picrate and hydrochloride[3] prepared by the method of Windaus and Langenbeck (*6*).

A sample of the picrate was oxidized to carbon dioxide and assayed as $BaCO_3$ in the Geiger counter to give a value of 400 cpm/mg of carbon (calculated to infinite thickness) for the picrate. This corresponds to 1,000 cpm/mg of carbon for the free base.

To 5 rats of 155 g average weight was administered subcutaneously a total of 0.92 g of the 4(5)-amino-5(4)-imidazolecarboxamide hydrochloride in 45 ml of solution half isotonic with respect to sodium chloride. Each rat received 1 ml of the solution three times a day for 3 days. The rats were kept in individual metabolism cages, and 24-hr collections of urine were pooled and kept in the cold room. The respiratory CO_2 contained negligible radioactivity.

The rats were killed on the fourth day (15 hr after the last injection), and the free nucleotides and the nucleic acids isolated by the procedure used in this laboratory. Adenine from the nucleotides and the 2 purines from the nucleic acids were isolated as the picrates, recrystallized, and converted to the hydrochlorides by suspending in acetone and saturating rapidly with dry HCl. Each fraction was separately purified by the chromatographic procedure of Cohn (*7*). The fractions were followed spectrophotometrically by comparing ratios of readings at 262 mμ and 248 mμ. A middle fraction containing a

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[2] We are indebted to K. B. Streeter, Edward A. McFadden, and Joyce L. Pyett, of the Organic Research Laboratory of Sharp & Dohme, Inc., for these analyses.

[3] We are indebted to Everett M. Schultz, of the Organic Research Laboratory of Sharp & Dohme, Inc., for a sample of the hydrochloride prepared by the method of Windaus and Langenbeck.