sporin C, produced as much enzyme as the parental strain. We have no evidence for any causal relation between the purine auxotrophy of this mutant and the reduced-temperature sensitivity of its penicillinase-repression system. Five other mutants requiring either a purine or pyrimidine base responded to heat like the parental strain.

The properties of the penicillinase system described in this report should be useful in the further study of the physiology and genetics of the regulation of this enzyme (*12*).

Note added in proof. Recent work indicates that the special properties of the 55C1-P1 mutant are related to its auxotrophy. Penicillinase formation in this strain is derepressed by heat in cells grown in nutritionally optimal, but not in suboptimal, concentrations of adenosine and guanosine. Failure to obtain derepression in cells grown in tryptic digest broth (Table 1) is due to this mechanism, as shown by increased growth rate and derepression in cells grown in broth supplemented with adenosine and guanosine.

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References and Notes

1. L. H. Geronimus and S. Cohen, *J. Bacteriol.* **73**, 28 (1957); R. P. Novick, *Biochem. J.* **83**, 229 (1962); E. L. Swallow and P. H. A. Sneath, *J. Gen. Microbiol.* **28**, 461 (1962); A. K. Saz and D. L. Lowery, *Biochem. Biophys. Res. Commun.* **15**, 525 (1964); A. K. Saz, N. Citri, D. L. Lowery, *Bacteriol. Proc.*, abstr. No. P42 (1965), p. 80.
2. F. Leitner and S. Cohen, *J. Bacteriol.* **83**, 314 (1962).
3. F. C. Neidhardt, in *Progress in Nucleic Acid Research and Molecular Biology*, J. N. Davidson and W. E. Cohn, Eds. (Academic Press, New York, 1964), vol. 3, p. 145.
4. T. Horiuchi, S. Horiuchi, A. Novick, *J. Mol. Biol.* **3**, 703 (1961).
5. A. Novick, E. S. Lennox, F. Jacob, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 397 (1963); J. Gallant and R. Stapleton, *Proc. Nat. Acad. Sci. U.S.* **50**, 348 (1963).
6. C. J. Perret, *Nature* **174**, 1012 (1954).
7. R. P. Novick, *J. Gen. Microbiol.* **33**, 121 (1963).
8. M. H. Richmond, M. T. Parker, M. P. Jevons, M. John, *Lancet* **1964-I**, 293 (1964).
9. The staphylococci used in this study were, for the most part, recent isolates from patients at Michael Reese Hospital. The possibility is not excluded that some strains may be duplicates although phage typing, as yet incomplete, indicates that most are different. We thank the Bacteriology Laboratory, Illinois Public Health Department, Chicago, for the phage typing.
10. A. Carere and I. Spada-Sermonti, *J. Bacteriol.* **88**, 226 (1964). We are indebted to Robert Fantl for isolation of these mutants.
11. R. C. Wood and E. Steers, *J. Bacteriol.* **77**, 760 (1959).
12. Supported in part by PHS grant AI-02457-07 from NIH. We thank Dr. R. P. Novick for supplying strains 8325 (*aw*) and 8325 (*air*).

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Biosynthesis of Vitamin A with Rat Intestinal Enzymes

Abstract. *Vitamin A is synthesized from β-carotene in cell-free homogenates of rat intestinal mucosa, the biosynthetic enzymatic activity being present in the soluble protein fraction of the homogenate. Also required are a heat-stable factor in the particulate fraction, molecular oxygen, and bile salts. The reaction is stimulated by glutathione. The product, obtained in yields of up to 50 percent, has been identified as vitamin A aldehyde (retinal) by way of its semicarbazone derivative. The reaction mechanism involves the central cleavage of β-carotene into two molecules of retinal.*

The conversion of β-carotene to vitamin A was first demonstrated by Moore, who fed β-carotene to vitamin A–deficient rats (*1*). Since then it has been established that the intestine is the major site of this conversion. Thus, retinyl ester (*2*), but not β-carotene, appears in the mesenteric or thoracic-duct lymph of rats and pigs given test meals of β-carotene (*3, 4*). Retinyl ester predominates in the thoracic-duct lymph of human subjects fed test meals of labeled β-carotene (*5*). The conversion of labeled β-carotene into retinyl ester has also been demonstrated during experiments with isolated rat intestinal loops in vivo and during studies in vitro with rat intestinal slices (*6*).

Studies on the mechanism of vitamin A biosynthesis have been greatly limited by the lack of a cell-free system capable of mediating the conversion of β-carotene to vitamin A. Information available on the reaction mechanism and other details of retinol formation has been reviewed (*7*). The two major hypotheses have continued to be: (i) central fission of β-carotene resulting in two molecules of vitamin A, and (ii) oxidative attack at a site other than the central double bond of β-carotene, with the eventual formation of one molecule of vitamin A per molecule of carotene.

We now report the active conversion of β-carotene to retinal with cell-free homogenate fractions from rat intestinal mucosa. Mucosal scrapings of rat duodenum and jejunum were homogenized, in a Potter-Elvehjem homogenizer with a loose-fitting teflon pestle, in 0.1*M* potassium phosphate buffer, *p*H 7.7, containing 30 m*M* nicotinamide and 4 m*M* $MgCl_2$. After centrifugation of the homogenate at 2000*g* for 20 minutes, the supernatant fraction (designated S-2) showed enzymatic activity for the conversion of β-carotene to retinal. Further centrifugation of the S-2 at 104,000*g* for 60 minutes resulted in the separation of soluble and particulate fractions, both of which were almost completely inactive when tested individually (*8*). The combination of soluble plus particulate fractions, however, was fully as active as the unfractionated S-2.

The activity of the combined soluble and particulate fractions was only slightly reduced after heating the particulate fraction at 95° to 100°C for 20 minutes. There was complete loss of enzymatic activity after heating the soluble fraction at 64°C for 55 seconds. The soluble fraction contains the enzyme (or enzymes) responsible for retinal biosynthesis, and this enzyme has been partially purified by precipitation with ammonium sulfate between 25 and 45 percent saturation. The particulate fraction appears to provide a necessary factor which is not an enzyme. This factor can be extracted from the particulate fraction with 90 percent acetone; it has not yet been identified.

The enzyme preparations were incubated with C^{14}-β-carotene, produced biosynthetically from C^{14}-acetate by the fungus *Phycomyces blakesleeanus*, as substrate. The reaction was allowed to proceed in the dark at 37°C with room air as the gas phase. At the end of 1 to 2 hours the incubation mixtures were extracted with 20 volumes of chloroform-methanol (2:1, by volume), and then 5 volumes of 0.01*N* H_2SO_4 were added. The lower chloroform phase was collected and evaporated, and the total lipid extract so obtained was chromatographed on columns of alumina (Woelm, activity grade III). Five fractions were collected, containing, respectively, β-carotene, retinyl esters, retinal, retinol, and more polar compounds including retinoic acid (*4, 9*).

With the complete system as described in Table 1, β-carotene can be converted to retinal with a usual yield of 30 to 50 percent. There was an absolute requirement for molecular oxygen and an almost absolute require-

Table 1. Cofactor requirements for the biosynthesis of vitamin A. The complete system contained 11 mg of soluble protein (104,000g supernatant), and an equivalent amount of washed cell particles (sedimented between 2000g and 104,000g); 200 μmole of potassium phosphate buffer, pH 7.7; 36 μmole of nicotinamide; 4.8 μmole of $MgCl_2$; 12 μmole of sodium taurocholate; and 10 μmole of glutathione. α-Tocopherol (1 mg) was added in solution in 25 μl of acetone, and then substrate C^{14}-β-carotene (0.7 μg, about 1100 count/min) in 50 μl of acetone was added. Final volume, 2 ml. Fx, fraction (after column chromatography); EDTA, ethylenediaminetetraacetate.

Omission from or addition to complete system	Distribution of C^{14} after 75 min incubation (percent)				
	Fx 1 (β-carotene)	Fx 2 (retinyl ester)	Fx 3 (retinal)	Fx 4 (retinol)	Fx 5+6 (polar; acids)
None	47	1	45	4	4
Minus enzyme*	87	2	5	3	2
Minus O_2 (argon as gas phase)	89	2	3	3	4
Minus bile salt	83	2	9	3	3
Minus glutathione	64	2	25	4	5
Plus EDTA (20 μmole)	51	2	38	4	5
Plus ascorbic acid (20 μmole)	44	2	43	5	8

* Minus both the soluble protein and the cell particles.

ment for bile salt for the conversion of β-carotene to retinal. The requirement for bile salt was relatively nonspecific since comparable yields of retinal have been obtained with sodium glycocholate (6 mM), cholate (6 mM), and deoxycholate (3 mM). Sodium dehydrocholate or lithocholate were ineffective in this system. The system was stimulated substantially by the addition of glutathione and was not affected by the addition of ethylenediaminetetraacetate (10 mM) or ascorbic acid (10 mM). There was no requirement for the addition of reduced or oxidized pyridine nucleotides.

The product of the reaction has been identified as retinal by addition of pure unlabeled retinal, followed by formation of the semicarbazone derivative (*10*). This was recrystallized four times without significant change in its specific radioactivity. The melting point of the recrystallized derivative was 189° to 190°C. Eighty-five percent of the radioactivity in column fraction 3 was established to reside in retinal by this procedure, after an incubation in which 29 percent of the radioactivity was recovered in fraction 3 (retinal) after column chromatography.

Calculations based on the stoichiometry of our results indicate that most of the reaction-product retinal must have arisen by central cleavage of the substrate β-carotene into two molecules of retinal. Thus, in one experiment three incubations were conducted without enzyme or with inactivated enzyme, and four incubations were conducted simultaneously with active enzyme. The same amount of substrate was added to each of the seven incubation flasks. After incubation, 1029 ± 13 count/min (*11*) were recovered in column fraction 1 (carotene) and 55 ± 9 count/min in fraction 3, from the three flasks without active enzyme. After incubation with active enzyme, 535 ± 28 count/min were recovered in fraction 1 and 460 ± 20 count/min in fraction 3. These results are not consistent with the possibility that only one molecule of retinal is formed from one molecule of β-carotene.

During the biosynthesis of vitamin A, therefore, β-carotene presumably reacts with molecular oxygen, and this reaction is followed by the cleavage of the central double bond to form two molecules of retinal. Retinal is then reduced to retinol, which is subsequently esterified, mainly with palmitic acid (*4, 5*). Glover, Goodwin, and Morton have demonstrated the ready reduction of retinal to retinol by the intestinal wall in vivo (*12*), and experiments in our laboratory have demonstrated that this reaction is mediated by a soluble protein, in the intestinal mucosal homogenate, which requires the reduced form of nicotinamide adenine dinucleotide as cofactor. The newly formed retinyl ester is then mainly incorporated into lymph chylomicrons (*4, 5*) and is subsequently transported by way of the intestinal lymphatics, to eventually enter the vascular compartment.

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References and Notes

1. T. Moore, *Biochem. J.* **24**, 692 (1930).
2. The terms retinol, retinal, retinyl ester, and retinoic acid refer, respectively, to vitamin A alcohol, aldehyde, ester, and acid.
3. S. Y. Thompson, R. Braude, M. E. Coates, A. T. Cowie, J. Ganguly, S. K. Kon, *Brit. J. Nutr.* **4**, 398 (1950); H. Wagner, F. Wyler, G. Rindi, K. Bernhard, *Helv. Physiol. Acta* **18**, 438 (1960).
4. H. S. Huang and DeW. S. Goodman, *J. Biol. Chem.* **240**, 2839 (1965).
5. DeW. S. Goodman, R. Blomstrand, B. Werner, H. S. Huang, T. Shiratori, *J. Clin. Invest.* **44**, 1054 (1965).
6. J. A. Olson, *J. Biol. Chem.* **236**, 349 (1961); and *J. Lipid Res.* **5**, 402 (1964).
7. T. Moore, *Vitamin A* (Elsevier, New York, 1957), p. 180; J. Glover, *Vitamins and Hormones* **18**, 371 (1960); J. A. Olson, *J. Lipid Res.* **5**, 281 (1964).
8. When tested alone, the particulate fraction was completely inactive, and the soluble fraction was only slightly active (usually about 10 percent as active as the unfractionated S-2).
9. For details of the chromatographic separations, and of the methods used to identify the compounds in the various fractions, see (*4*) and DeW. S. Goodman, H. S. Huang, T. Shiratori, *J. Lipid Res.* **6**, 390 (1965).
10. C. D. Robeson, W. P. Blaus, J. M. Dieterle, J. D. Cawley, J. G. Baxter, *J. Amer. Chem. Soc.* **77**, 4120 (1955).
11. Mean ± standard error of the mean for the individual incubations. The C^{14} radioactivity (count/min) was measured in a Packard Tri-Carb liquid scintillation counter, with an efficiency of 55 percent.
12. J. Glover, J. W. Goodwin, R. A. Morton, *Biochem. J.* **43**, 109 (1948).
13. Supported by NIH grant AM-05968. We thank T. Shiratori and Joan Vormbaum for assistance. D.S.G. has a Career Scientist Award from the Health Research Council of the City of New York under contract 1-399. H.S.H. is a trainee in Metabolism and Endocrinolgy, grant T1-AM-5397 from National Institutes of Health.

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Trend Curves of the Rate of Species Description in Zoology

Abstract. *Trend charts of approximately 0.4 percent of the animal kingdom indicate that the describing and naming of species, "alpha taxonomy," is far from completed.*

Abelson (*1*) expresses a widely held opinion in saying that "work on the descriptive features of gross morphology has largely been completed, and the rate of discovery and description of new species has slackened." Blackwelder (*2*), however, says that "it must be recognized that the job of making known the animals of the earth is so far from finished that we don't yet know even the general pattern of relative abundance of all the major groups." The conflict here calls for some kind of factual background that so far seems to be lacking.

A few years ago I started to compile numerical data concerning the rate of description of species in some insect groups. The results were so encourag-