lysozyme and thus further exposes the protoplasmic contents of the cell to the direct action of the proteolytic enzyme.

Summary. It has been shown that *S. aureus* grown in the presence of subinhibitory concentrations of nafcillin produced a disturbance or disorganization of the cell wall fabric resulting in a marked accumulation of a polysaccharide within the cell. An appreciable polysaccharide response was also found for oxacillin, methicillin and erythromycin. This conclusion is based on data which demonstrate a clear differentiation between such an inhibitor of bacterial wall synthesis as penicillin which exerted a pronounced effect on polysaccharide synthesis and other antibacterial antibiotics which failed to increase the quantity of polysaccharide formed. In addition, treatment with nafcillin rendered the normally lysozyme resistant cells of *S. aureus* susceptible to partial lysis by lysozyme and the concomitant addition of trypsin resulted in further dissolution of the cell bodies. Electron micrographs of such treated cells subjected to thermal stress provide further evidence of the relationship between accumulation of polysaccharide and actual interference with cell wall synthesis. The significance of the results relating to the mechanism of action of the semi-synthetic penicillin nafcillin and possibly other penicillins is discussed.

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Asymmetrical Incorporation of C^{14} Acetate into β-Carotene Biosynthesized by *Phycomyces blakesleeanus.** (28701)

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Lotspeich *et al*(1) reported on the partial incorporation of C-1 and C-2 labeled acetate into the side chain of β-carotene by *Phycomyces blakesleeanus.* The evidence at that time indicated that the carbons of the ring system were more radioactive than those of the side chain. Furthermore, when C-2 acetate was utilized, radioactivity was found in the positions expected if carotene was formed by a head to tail condensation of acetate, as well as in the other positions. This was in contrast to the finding of Grob and Butler (2) that β-carotene synthesized by *Mucor hiemalis* from C-2 acetate contained activity only in positions expected if β-carotene was formed by a head to tail condensation of acetate. Goodwin's results(3) with *Phycomyces blakesleeanus* were similar to those of Grob and Butler(2).

In this paper we describe the incorporation of C-1 and C-2 labeled acetate into the ring system of β-carotene.

Materials and methods. Radioactive substrates 1-C^{14} acetate and 2-C^{14} acetate were obtained from commercial sources.

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Culture methods: The culture methods used were the same as described by Lilly *et al*(4) with the exception of one gram of magnesium sulfate and 0.083 g of potassium acetate added per liter of medium.

Isolation and degradation of β-carotene: β-carotene was isolated as described by Lilly *et al*(4) and chromatographed on silicic acid. The silicic acid was prepared by suspending 115 g of silicic acid (Mallinckrodt 100 Mesh) in 600 ml of water containing 15 g of NaOH. The material was filtered, washed with 100 ml of water and dried at 45°C for 48 hours. Twenty-five g of this silicic acid was suspended in petroleum ether (B.P. 37°C) and poured into a column 2.5 × 42.6 cm long. The column was placed under a positive nitrogen pressure and the silicic acid packed to a height of 17.2 cm.

Two mg of commercial carotene dissolved in the minimum amount of petroleum ether was placed on the column and eluted in order to remove any oxygen which might be entrapped in the silicic acid. The carotene extract of *Phycomyces blakesleeanus* dissolved in the minimum amount of petroleum ether then was placed on the column, and eluted with petroleum ether at the rate of 5 ml/min. The major orange band was collected, evaporated to dryness under nitrogen, and washed with approximately 50 ml of methanol. The β-carotene crystals which broke loose from the side of the flask during the washing process were filtered on a sintered glass filter, dissolved in petroleum ether, added to the methanol washed carotene and taken to dryness. This procedure was repeated 8 times. The resulting all trans-β-carotene had $E^{1\%}_{1\,cm}$ values of 2600 or greater in 12 cases. In the majority of cases the $E^{1\%}_{1\,cm}$ value was 2650. Chromatography of the resulting β-carotene over magnesium oxide and aluminum oxide revealed no impurities. The ultraviolet, visible, and infrared spectrum of the β-carotene was identical with that of a pure synthetic sample obtained from Hoffmann-LaRoche Inc.

The β-carotene was degraded according to the procedure of Steele and Gurin(5) using a chromic acid oxidizing medium. The initial oxidations were carried out in a Nuclear of Chicago oxidation train and the resulting carbon dioxide collected in an evacuated ionization chamber and counted. The mono and dicarboxylic acids resulting from this oxidation were separated on a silicic acid column as described by Steele and Gurin(5). A weighed portion of each acid was completely oxidized to carbon dioxide and counted while the remainder was decarboxylated with hydrazoic acid in concentrated sulfuric acid (Schmidt reaction) and the resulting carbon dioxide determined. The amount of carbon dioxide produced by the Schmidt reaction was measured in a manometric Van Slyke apparatus(6) and radioactivity determined in a Nuclear of Chicago Dynacon.

Results and discussion. The method which we have used for purification of β-carotene significantly reduced the time and work previously required to purify β-carotene biosynthesized by *Phycomyces blakesleeanus,* and has resulted in higher yields.

Initially we used both the method of Steele and Gurin(5) and the method of Grob and Butler(7) for the degradation of β-carotene. However, the method of Steele and Gurin gave a more complete analysis of the β-carotene and resulted in higher yields. Consequently, the results reported here are limited to the method of Steele and Gurin.

Five degradation experiments were carried out on β-carotene biosynthesized by *Phycomyces blakesleeanus* from 1-C^{14} and 2-C^{14} acetate, respectively. However, only the results of one experiment in each case are listed in Table I since similar experiments yielded practically identical results. The specific activities listed for the various carbon atoms in Table I include the carbons of both halves of the symmetrical β-carotene molecule since it is presumed that both halves are labeled.

The results recorded in Table I, showing the incorporation of the carboxyl carbon of acetate into positions 1, 1′, 3, 3′, 5, 5′ and the incorporation of the methyl group of acetate into all positions of the ring system of the carotene molecule, confirm our previous work involving the side chain. These results are also in agreement with those of Steele and Gurin(5) using *Euglena gracilis,*

and support the theory that the citric acid cycle is operative in *Phycomyces blakesleeanus*. This work also substantiates our previous speculation that the ring carbons of the carotene molecule are more radioactive than those of the side chain. Thus, the evidence seems to indicate a difference in incorporation of acetate into the side chain and the ring under the experimental conditions which we are using for the biosynthesis of β-carotene.

Summary. β-carotene biosynthesized by *Phycomyces blakesleeanus* was purified by column chromatography over basic silicic

TABLE I. Partial Distribution of Activity in β-Carotene Biosynthesized by *Phycomyces blakesleeanus* from Glucose and Labeled Acetate.

Compound degraded	Carbon atoms involved	Precursor 1-C^{14}-acetate			Precursor 2-C^{14}-acetate		
		Wt sample, mg	Activity sample, dpm	d/m/μmole C	Wt sample, mg	Activity sample, dpm	d/m/μmole C
β-Carotene (oxidation)	40	52.15	152,600	39	50.93	322,000	85
Partial oxidation‡	C-7,8,10,11,12,14,15	56.31	29,450	23	57.3	60,000	46
Geronic acid oxidation	C-1,2,3,4,5,6, C-5 methyl	2.53	7,450	57	4.165	28,190	131
Schmidt*	C-5 methyl	.572	60	4.6	.518	1,342	114
Schmidt	C-6	.867	98	5	.953	2,775	128
Dimethyl adipic acid (oxidation)	C-1,2,3,4,5,6	1.86	5,400	63	4.513	27,205	131
Schmidt	C-5,6	.685	1,170	75	.753	2,390	140
Dimethyl glutaric acid (oxidation)	C-1,2,3,4,6	3.45	7,700	51	3.400	18,850	127
Schmidt	C-4,6	1.13	150	5.7	1.050	3,030	127
Dimethyl succinic acid (oxidation)	C-1,2,3,6	1.38	3,339	59	.954	5,040	129
Schmidt	C-3,6	.715	1,380	85	.680	1,984	128
Dimethyl malonic acid (oxidation)	C-1,2,6	1.12	1,610	38	1.231	6,050	130
Sulfuric acid†	C-2,6	.613	83	6	.556	1,570	124
Oxidation†	C-1 methyl	.231	25	5	.354	1,055	131

* Three and one-half equivalents of hydrazoic acid react with geronic acid to produce acetic acid from carbon 5 and the attached methyl group. Decarboxylation of this acetic acid by the Schmidt reaction yields the methyl carbon as methylamine which was oxidized(5).

† Dimethyl malonic acid was decarboxylated in sulfuric acid to CO_2 and isobutyric acid. This CO_2 was from carbons 2 and 6. The isobutyric acid was oxidized to acetic acid and the acetic acid reacted with hydrazoic acid to yield the C-1 methyl group as methylamine(5).

‡ Partial oxidation of β-carotene was achieved with potassium dichromate(5).

acid. Degradation studies on the ring system of this radioactive β-carotene indicate that the carboxyl carbon of acetate is incorporated into positions 1, 1′, 3, 3′, 5 and 5′ of the ring system whereas the methyl carbon is incorporated into all positions of the ring system. Our studies also indicate that the ring carbons are more radioactive than the side chain carbon atoms.

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Serum Sialic Acid Levels in Experimental Anaplasmosis.* (28702)

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Members of the sialic acid group of compounds occur in various tissues and body fluids(2,12), viz., mucous secretions, urine, egg albumin, milk, cerebrospinal fluid, blood serum, salivary gland, and brain. Sialic acid is the group name for several compounds of substituted neuraminic acid derivatives (N-acetyl, N-glycolyl, N,O-diacetyl-neuraminic acid). The unsubstituted 9-carbon compound is called neuraminic acid(3). As may be seen from Fig. 1, N-acetyl neuraminic acid (NANA) may be visualized as an aldol condensation product of N-acetyl hexosamine and pyruvic acid(12). On deacetylation, NANA yields neuraminic acid(4). In nature, sialic acids occur as components of mucoproteins(2).

Sialidase (neuraminidase) is an enzyme present in bacteria and in myxoviruses. Its action upon receptors of tissue cells, which are mucoprotein in nature, results in liberation of sialic acid. It is believed that the activity of sialidase serves to attach the virus particles to the erythrocytes or susceptible host cells as a first step in the invasion of the latter cells(4).

Anaplasma marginale, the etiologic agent of anaplasmosis, is considered a rickettsial parasite of erythrocytes of cattle(5). Anaplasmosis is an infectious and transmissible disease of cattle manifested by progressive anemia associated with the presence of intraerythrocytic inclusions of *A. marginale* (Fig. 2). Electron microscopic and fluorescent antibody studies of *A. marginale* have provided static evidence that the initial bodies of this parasite are capable of attachment to, and penetration of, the erythrocytic membrane(6). Therefore, it was of interest to know whether the sialic acid of the mucoproteins of the receptors of erythrocytes is

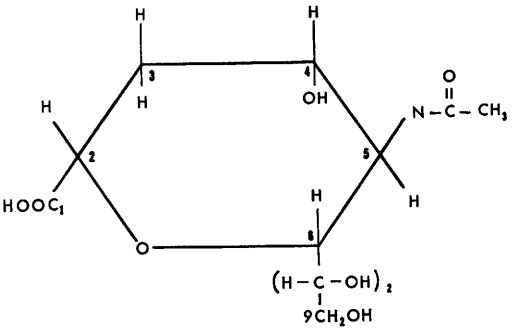

FIG. 1. Probable structure of N-acetyl neuraminic acid (after Whitehouse, M. W., Zilliken, F., *Methods of Biochemical Analysis*, v8, 199. Ref. 12.).

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