purified. Further purification, however, will be necessary to establish unequivocally whether cobalt is a constituent of the enzyme. The addition of graded amounts of cobalt or vitamin B_{12}, to extracts prepared from cobalt-deficient cells or to purified fractions of the enzyme, did not activate nitrate reductase. The micronutrient was readily dialysed from the purified enzyme without an apparent loss of activity. These preliminary results suggest that cobalt is unlikely to be a functional constituent of nitrate reductase and that its effect on the enzyme may well be on its formation rather than on its action. This concept is further supported by the finding that the uptake of nitric oxide is also diminished by cobalt deficiency. Further work is in progress to elucidate the possible function of cobalt in the utilization of nitrate in the Rhizobia.

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Intracellular distribution of sialic acid and its relationship to membranes

In "sialopolymers" such as glycoproteins and glycolipids, sialic acid is almost always found terminally bound, and in certain tissues such as red blood cells it is considered to exist on the periphery of the cell. It was therefore of interest to determine the distribution of sialic acid in subcellular fractions of liver, especially with regard to the particulate membranes.

Excised livers from 250–300 g male, Wistar rats were perfused with cold 0.25 *M* sucrose and homogenized and fractionated according to the procedure of SCHNEIDER[1], except that the microsomes were obtained by centrifugation at 105 000 × *g* for 1 h in a Spinco preparative ultracentrifuge (Model L). Each particulate fraction was washed twice by resuspension and centrifugation. Preparations of mitochondrial

membranes were obtained by sonication according to the method of LEHNINGER, SUDDUTH AND WISE[2]. Microsomal membranes were obtained in "soluble" form by the method of KIRSCH, SIEKEVITZ AND PALADE[3]. The membrane preparations were then dialyzed 16 h against 12 l of distilled water and lyophilized. The dried residues were either hydrolyzed in 0.1 N H_2SO_4 for 1 h at 80° or extracted with organic solvents and then hydrolyzed. Sialic acid released by hydrolysis was isolated by Dowex-1 (acetate) column chromatography as described by SVENNERHOLM[4], the eluates being assayed for sialic acid by the 2-thiobarbituric acid method of WARREN[5]. Protein nitrogen was determined by the LOWRY method[6] with bovine serum albumin (Armour) as standard.

As shown in Table I, sialic acid is distributed throughout the cell, with the exception of the nuclear fraction. The highest concentration is in the microsomal and supernatant fractions. The sialic acid in the supernatant fraction is in bound form, as indicated by a negative thiobarbituric acid test prior to hydrolysis and by the inability of the Dowex-1 column to remove the sialic acid from unhydrolyzed samples. The sialic acid to protein ratio in the microsomes is twice that in the other fractions. A few preliminary experiments on mitochondrial and microsomal "membranes" have been carried out. Although the yield of membranes varied greatly, the sialic acid–protein ratio for a given type of particle was relatively constant, a finding suggesting that the amino sugar is an integral part of the mitochondrial and microsomal membranes.

TABLE I

INTRACELLULAR DISTRIBUTION OF SIALIC ACID IN RAT LIVER

The values of protein N and sialic acid (as N-acetylneuraminic acid) are per g liver, wet wt. The values represent the means ± the standard error of the mean.

Fraction	*Number of animals*	*Protein N (mg/g)*	*Sialic acid (μg/g)*	*Sialic acid/protein N**
Homogenate	7	28.84 ± 1.24	275.80 ± 13.3	9.70 ± 0.21
Nuclear	5	5.74 ± 0.81	undetectable	—
Mitochondrial**	7	4.11 ± 0.38	24.38 ± 1.18	5.97 ± 0.93
Microsomal***	6	4.99 ± 0.45	103.20 ± 8.76	22.70 ± 4.81
Supernatant	5	9.88 ± 0.91	99.80 ± 20.1	9.98 ± 3.58
Total		24.72	226.8	
Recovery (%)		85.6	82.2	

* These values are averages of the sialic acid–protein-N ratios for the animals in each group
** Sialic acid–protein-N ratio in mitochondrial membranes, 4.70 ± 0.70 (2 experiments).
*** Sialic acid–protein-N ratio in microsomal membranes, 17.9 ± 1.52 (2 experiments).

The identification of the sialic acid in the particles was established not only by the column chromatographic procedure and thiobarbituric acid assay mentioned above, but also by its release by neuraminidase (Behringswerke), its reactivity in the resorcinol assay of SVENNERHOLM[7], and, in one experiment, its paper chromatographic behavior as N-acetylneuraminic acid.

SJÖSTRAND[8] has suggested that glycoprotein may be a constituent of the membranous material of mitochondria. WOLFE[9] has very recently reported that gangliosides are present in microsomes and in other apparently membranous fractions of

guinea-pig cerebral cortex. It will therefore be of considerable interest to characterize the sialic acid-containing constituents of the mitochondrial and microsomal membranes of rat liver. Preliminary experiments indicate that the sialic acid in these fractions is not extractable into organic solvents, even following digestion of the membranes with trypsin.

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The chemical structure of one component of clupeine*

Clupeine from Pacific herring (*Clupea pallasii*) has been fractionated on a preparative scale in two main fractions, Y and Z, by elution chromatography from alumina[1]. By means of counter-current distribution, the Y fraction was further resolved into two fractions, YI (amino acid composition: Arg, Pro, Ala, Ser, Thr, Ileu, and Gly; N-terminus: Ala $\gg$ Pro) and YII (composition: Arg, Pro, Ala, Ser, Val, and Thr; N-terminus: Pro $\gg$ Ala), while in contrast no further ıractionation was achieved with Z fraction (composition: Arg, Pro, Ala, Ser, and Val; N-terminus: Ala only). This fraction, which is probably homogeneous, has now been used for structural studies.

Amino acid analyses of clupeine Z using both the dinitrophenyl method and an automatic analyzer, gave the molecular formula $Ala_3Ser_3Pro_2Val_2Arg_{21}$. Only alanine was found at the N-terminus by isolation as the dinitrophenyl derivative and the measurement of the ratio of absorbancies at 390 mμ and 360 mμ of the dinitrophenyl-protein[2]. The molecular weight estimated from the amount of dinitrophenyl introduced (about 5000 as hydrochloride) was consistent with that calculated from the above formula (4929 as hydrochloride). Using a procedure practically the same as

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