A similar observation was made with *E. coli* 15 t^-h^-(thymine- and histidine-less). Cells labelled with nitrogen-15, grown in nitrogen-15-salts-glucose medium supplemented with ^{15}N-thymine (5 µgm. per ml., prepared from nitrogen-15-labelled cells of *E. coli* 15) and ^{14}N-L-histidine-hydrochloride (10 µgm. per ml.), were washed and transferred into nitrogen-14 medium with nitrogen-14-thymine, but no histidine, and incubated at 37° C. with shaking. After 120 min., DNA and RNA increased by 75 and 30 per cent, respectively, while the amount of protein and the viable cell number remained constant ($1 \cdot 5 \times 10^8$ per ml.)[6]. The sample taken after 120-min. incubation showed an intense ^{15}N–^{14}N hybrid band of DNA and a light ^{15}N–DNA band in the ultra-violet absorption photograph taken following density gradient centrifugation.

From these observations it is concluded that DNA formation involves replication in non-dividing, as well as in dividing, cells. This suggests the normality of the DNA formed without accompanying protein synthesis, which has been confirmed on biological grounds[7].

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DAISUKE NAKADA
FRANCIS J. RYAN

Department of Zoology,
Columbia University,
New York 27.

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Bovine Submaxillary Mucin

A CONVENIENT method for the isolation of bovine submaxillary mucin in a relatively undegraded and homogeneous state compared to earlier methods[1] has been developed in this Laboratory. Fresh glands (640 gm.) were ground and stirred mechanically with three changes of 500 ml. of cold water for a total of 54 hr. The pooled extracts were clarified by centrifuging at 26,360*g* for 30 min. and adjusted to *p*H 3·5. The resulting mucin clot was separated and dissolved in cold water by neutralization to 7·5, and lyophilized. The product was purified by solution in 500 ml. of cold 50 per cent (w/v) aqueous calcium chloride. After centrifugation at 26,360*g* for 30 min., the supernatant was fractionated with cold ethanol. The fraction separating at 60–75 per cent (v/v) ethanol was collected by centrifugation at 26,360*g* for 30 min., dissolved in water, dialysed against water and lyophilized. All procedures were carried out at 0–4° C. The yield was 0·64 and 2·5 per cent of the glands on fresh and dry weight basis, respectively. The recovery of sialic acid was 36 and 60 per cent of that in the extract and mucin clot, respectively. A repetition of the procedure did not alter the composition of the product. The lyophilized powder could be stored at 2–4° C. without change of its properties.

The mucin was soluble in water and gave clear, colourless, viscous solutions. The solutions (0·5 per cent) gave single peaks on electrophoresis and ultracentrifugation at *p*H 7·4 and 11·3. The electrophoretic mobility was $-6 \cdot 7 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ V.$^{-1}$ in phosphate buffer, *p*H 7·4 and ionic strength 0·1. The sedimentation constant ($S_{20,w}$) was 2·6 at 0·5 per cent in the same buffer. No decrease in relative viscosity was observed when that of the product was compared with that of the extract at the same concentration of sialic acid. The intrinsic viscosity of the product in phosphate buffer, *p*H 7·4 and ionic strength 0·1, was 13·4 dl./gm. The mucin contained 9·2 per cent nitrogen, 28 per cent sialic acid, 24 per cent hexosamine (as N-acetylgalactosamine), 2 per cent neutral sugars (as D-galactose), and 37 per cent protein. D-Galactose and L-fucose were the only neutral sugars detected on paper chromatograms. Tyrosine and sulphur-containing amino-acids were not found on the chromatograms.

The mucin (1 gm. in 200 ml.) at *p*H 7·4 was incubated at 37° C. with 50 mgm. of crystalline trypsin. A reduction of the viscosity to that of the solvent occurred within a few minutes. After 24 hr. the digest was dialysed with only a slight loss in protein. The tryptic digestion was repeated and the mucin was recovered from the digest by precipitation with ethanol in the presence of 5 per cent (w/v) calcium chloride. After a reprecipitation the final product was dissolved in water, dialysed, and then lyophilized. The yield was 80 per cent. Its electrophoretic pattern showed a single peak at *p*H 11·3 with unchanged mobility. It contained 8·7 per cent nitrogen, 31 per cent sialic acid, 27 per cent hexosamine, 2·5 per cent neutral sugars, and 32 per cent protein. The relative viscosity of a 0·5 per cent solution in phosphate buffer, *p*H 7·4 and ionic strength 0·1, was 1·16. These results indicate that the protein occupies an integral part of the mucin molecule and also is associated with its rheological characteristics.

An aqueous solution of the undigested mucin (1 per cent) was heated at 100° C. with hydrochloric acid. Nearly all the sialic acid became dialysable after treatment at *p*H 2·5 for 30 min., whereas practically no hexosamine was liberated. After 120 min., the hexosamine lost by dialysis was about 25 per cent, and no loss of protein was observed.

These results are compatible with one of the earlier structures proposed by Gottschalk[2] of a protein core surrounded with N-acetylgalactosamine units with all sialic acid as external end-groups.

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S. TSUIKI
Y. HASHIMOTO
W. PIGMAN

Arthritis and Rheumatism Research Laboratory,
University of Alabama Medical Center,
Birmingham, Alabama.

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