TABLE II

EFFECT OF INHIBITORS

Inhibitors were mixed with the enzyme before the addition of the other components of the assay as described in Table I and including 0.1 μmole vitamin K

Compound added	*Final concentration*	*% Inhibition*
KCN	10^{-3} *M*	100
α-α′ Dipyridyl	$6 \cdot 10^{-3}$ *M*	67
o-Phenanthroline	$5 \cdot 10^{-3}$ *M*	90
Na diethyldithiocarbamate	10^{-3} *M*	29
Salicylic acid	10^{-3} *M*	32
8-Hydroxyquinoline	10^{-3} *M*	29
Thiourea	10^{-3} *M*	41
2-Heptyl-4-hydroxyquinoline	$4 \cdot 10^{-4}$ *M*	95

Work is now in process to clarify this system and its physiological significance.

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Biosynthesis of hyaluronic acid by cell-free extracts of group-A streptococci*

Previous studies have demonstrated that both the glucosamine and glucuronic acid moieties of HA** synthesized by Group-A streptococci are derived from glucose[1]. The discovery of uridine nucleotides containing N-acetylated amino sugars[2] and glucuronic acid[3] and the established role of uridine nucleotides as glycosyl donors[4] suggested that these compounds are intermediates in the biosynthesis of mucopolysaccharides. CIFONELLI AND DORFMAN[5] have demonstrated the presence of UDPAG, UDPGA and other non-identified fractions containing uronic acid and N-acetylglucosamine in a strain of Group-A streptococcus grown under conditions optimal for the production of HA. The only direct evidence in support of the role of uridine nucleotides in acid mucopolysaccharide synthesis is given by the reports of GLASER AND BROWN[6,7] that extracts of Rous sarcoma incorporate into HA, ^{14}C from labeled UDPAG, AG-6-P+UTP, or UDPG. However, a large portion of the radioactivity was lost on reprecipitation or electrodialysis. No incorporation was obtained when labeled UDPGA was used.

It is the purpose of this communication to present evidence that a cell-free extract of a strain of Group-A streptococcus (A111, Type 18) incorporates glucoronic acid from UDPGA. This system has an absolute requirement for UDPAG and Mg^{++} and a relative requirement for AG-1-P and ATP.

Cells were grown as previously described[5], harvested by centrifugation at 30,000 × *g* for 10 min, and washed twice with 0.05 *M* phosphate buffer, pH 7.0. Following treatment in a

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** The following abbreviations are used in this paper: hyaluronic acid, HA; uridine diphospho-N-acetylglucosamine, UDPAG; uridine diphosphoglucoronic acid, UDPGA; uridine diphosphoglucose; uridine triphosphate, UTP; adenosine triphosphate, ATP; diphosphopyridine nucleotide, DPN; N-acetylglucosamine-1-phosphate, AG-1-P; N-acetylglucosamine-6-phosphate, AG-6-P.

Raytheon sonic oscillator at 9 kc, the sonicate was centrifuged at 10,000 × *g* for 10 min at 4° and the supernatant used. The UDPGA was isolated from another strain of streptococcus (Richards strain) by the method previously reported[5]. Tritiation was carried out by the method of WILZBACH[8] and repurification by column chromatography using the neutral ammonium acetate–ethanol solvent of PALADINI AND LELOIR[9]. The repurified material showed the same activity as a glucuronyl donor with *p*-nitrophenol as the highly purified starting material[10]. The AG-1-P was prepared by the method of MALEY, MALEY AND LARDY[11]. UDPAG was obtained from the Sigma Chemical Company.

The HA from each sample was isolated and purified in the following fashion. After the indicated incubation time, 1.07 mg HA (isolated from the growth supernatant of the same organism by the method of CIFONELLI AND MAYEDA[12]) was added and the sample boiled for 5 min. Sufficient additional HA was added to make a total of 10.7 mg. After adjusting the volume to 10 ml the tubes were centrifuged for 20 min at 2000 r.p.m. The supernatant was dialyzed successively for 6 h against running tap water, 10 h against 15 l distilled H_2O, and finally for 24 h against 15 l distilled H_2O. The sample was centrifuged for 20 min at 30,000 × *g* and to the supernatant were added 1 drop 4 *N* NaOH and 1 drop phenolphthalein. The solution was brought just to the point of boiling, cooled, neutralized with 3–4 drops 1 *N* HCl, and passed over an 800-mg column of Norite-A–20% stearic acid. The latter treatment removes all ultraviolet-absorbing material. The HA was precipitated as the cetyl pyridinium complex by the addition of 1–2 drops 1 *M* Na_2SO_4 and 1.5–2.0 ml 1% cetyl pyridinium chloride. The complex was centrifuged, washed 3 times with H_2O, and dissolved in 5 ml methanol. Insoluble material was removed by centrifugation. The HA was precipitated from the supernatant with 1 vol. glacial acetic acid and washed 3 times with methanol, once with methanol–ether, twice with ether and dried in a vacuum desiccator over Drierite. The HA was dissolved in 1 ml 1 *M* hyamine[13,14] and the radioactivity determined in the Tri-Carb Liquid Scintillation Spectrometer, using 2,5-diphenyloxazole as a phosphor in toluene. HA prepared by this method showed a hexosamine : uronic acid : N molar ratio of 1 : 0.99 : 0.9.

The results of a typical experiment are illustrated in Table I. Omission of $MgCl_2$ or UDPAG resulted in failure of incorporation of radioactivity while omission of AG-1-P or ATP caused a significant diminution.

When the isolated HA was repurified by adsorption and elution from Dowex-1 Cl^-, no change in specific radioactivity was observed.

In other experiments employing the same components as above, but utilizing AG-1-P as the radioactive component, radioactive HA was obtained. Under these conditions the UDPGA could be replaced by UDPG and DPN. The inclusion of UTP in the presence of AG-1-P abolishes the absolute requirement for UDPAG.

In one experiment utilizing an enzyme obtained from a 30-min sonicate (Table I), 12.5% of uronic acid of the UDPGA was incorporated into HA. This calculation was based on the assumption that the UDPGA was uniformly labeled. Correction for the presence of HA in the enzyme preparation results in an increase in the estimate of incorporation.

TABLE I

INCORPORATION OF ^3H FROM ^3H-UDPGA INTO HA

All tubes contained a total volume of 1.5 ml including 0.75 ml of enzyme with a final phosphate concentration (pH 7.0) of 0.05 *M*. 10.7 mg carrier HA was added to each tube. The ^3H-UDPGA used had an activity of 580,000 counts/min/μmole.

Time min	*^3H-UDPGA μmole*	*UDPAG μmole*	*N-Ac-Gm-1-P μmole*	*$MgCl_2$ μmoles*	*ATP μmole*	*Radioactivity counts/min/mg HA*
0	1.0	1.0	1.0	10.0	1.0	0
130	1.0	1.0	1.0	10.0	1.0	820
130	1.0	1.0	0	10.0	1.0	610
130	1.0	0	1.0	10.0	1.0	3
130	1.0	1.0	1.0	0	1.0	15
130	1.0	1.0	1.0	10.0	0	600
130*	1.0	1.0	1.0	10.0	1.0	2600

* The enzyme used in this preparation was obtained from a 30-min sonicate, while that used in all other test combinations was from a 15-min sonicate.

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The role of deoxyribonucleic acid in ^{32}P incorporation into isolated nuclei *

The importance of deoxyribonucleic acid (DNA) in the synthesis of nuclear protein and ribonucleic acid (RNA) has been demonstrated, but its action does not seem to be very specific[1,2]. Since different workers have observed the incorporation of various precursors into DNA in isolated nuclei[3–5], we have undertaken to clarify the role of pre-existing DNA in DNA synthesis with rabbit appendix nuclei.

Nuclei isolated from the appendix and Peyer's patch of adult rabbits by a slight modification of the method of ALLFREY *et al.*[1] were incubated with ^{32}P-orthophosphate according to FRIEDKIN AND WOOD[4]. The procedure of digestion with pancreatic deoxyribonuclease (DNase) and subsequent treatment of nuclei were essentially similar to the ones employed by ALLFREY *et al.*[1]. 1 ml of incubation mixture containing about $3 \cdot 10^8$ nuclei and 10 μC ^{32}P were shaken at 37° for 90 min. The acid-soluble organic-phosphate fraction (OASP) was obtained according to HOKIN AND HOKIN[6]. Nucleic acids were extensively purified by the routine method used in this laboratory[7]. Radioactivity incorporated into RNA and DNA was evenly distributed among various nucleotide fractions obtained chromatographically (Dowex-1, formic acid systems) following alkaline hydrol-

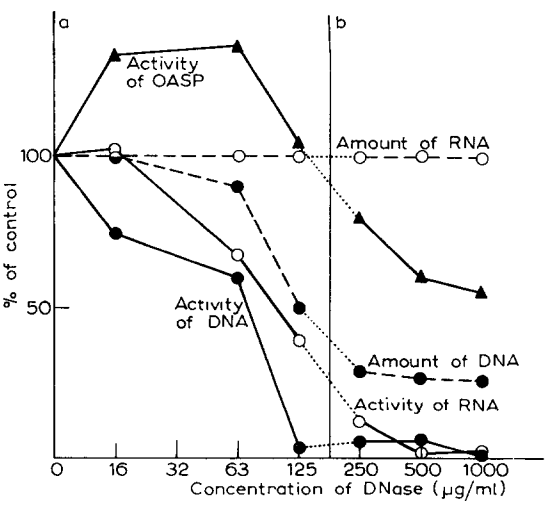

Fig. 1. The effect of DNase treatment of isolated nuclei. Activity refers to the total radioactivity (counts/min/tube). With nucleic acids, this was calculated from the specific activity (counts/min/μg P) of purified samples and the total amount (μg P/tube) determined by the conventional Schneider method. Specific activities of OASP, RNA and DNA of untreated control nuclei were 1076, 32, 2.4 (Expt. a) and 759, 43, 0.8 (Expt. b).

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