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Uridine, Cytidine, and Deoxyuridine Derivatives*

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5-(*p*-Chlorobenzylidene)-aminouridine, 5-(2′,4′-dichlorobenzylidene)-aminodeoxyuridine, 5-(3′,4′-dichlorobenzylidene)-aminouridine, 5-(3′,4′-dichlorobenzylidene)-aminodeoxyuridine, 5-bromoacetamidouracil, and 5-hydroxycytidine were synthesized, and the inhibitory effects of these compounds on growth of wild-type Neurospora and *Escherichia coli* K-12 were determined. All of the Schiff's base derivatives inhibited growth of Neurospora. Bromoacetamidouracil and hydroxycytidine were inactive. All the new compounds completely inhibited growth of *E. coli*. Inhibition reversal studies in *E. coli* indicate that amino acid metabolism, rather than nucleic acid metabolism, is involved in the inhibitory effects of the aminouridine Schiff's bases. The corresponding aminodeoxyuridine derivatives interfere with nucleic acid metabolism in *E. coli*.

The hydrogen at the 5 position of uracil and cytosine may be displaced readily by other substituents with intact pyrimidine or pyrimidine nucleosides used as starting materials. The 5 position is also the locus of numerous substitutions which take place biologically, such as the formation of thymine (Friedkin and Kornberg, 1957), hydroxymethylcytosine (Flaks and Cohen, 1959), methylcytosine (Johnson and Coghill, 1925), and pseudouridine (Cohen, 1959; Davis and Allen, 1957). This combination of circumstances has resulted in the synthesis of a number of 5-substituted derivatives which have been shown to produce a variety of interesting biological effects involving the formation or function of naturally occurring pyrimidine compounds (Handschumacher and Welch, 1960). This communication described the synthesis and biological activity of additional 5-substituted pyrimidine nucleosides.

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The choice of chemical groups to be substituted at the 5 position was based on a desire to prepare compounds which might be useful for correlating the chemical nature of the 5 substituents with biological activity. Some substituents were selected because of their similarity to compounds previously shown to be interesting antimetabolites. Others were prepared as potential alkylating agents which retain structural characteristics of nucleic acid precursors. The derivatives, 5-aminouridine and 5-aminodeoxyuridine, which have been shown to inhibit growth of Neurospora (Roberts and Visser, 1952b) and bacteria (Beltz and Visser, 1957), were used as convenient starting materials for the preparations of Schiff's bases.

The 5-benzylidineaminouracil derivatives were of interest because of their structural similarity to 5-(3′,4′-dichlorophenyl)-6-(ethyl)-2,4-diaminopyrimidine, an antitumor drug (Sugiura, 1955), and to 5-(*p*-chlorophenyl)-6-ethyl-2,4-diaminopyridmidine (Falco *et al.*, 1951), an antimalarial drug; the latter has been shown to interfere with folic acid metabolism (Hitchings, 1952). These

Schiff's bases are stabilized by the conjugate double bond relationship with the pyrimidine ring. 5-Bromoacetamidouracil was prepared because it was anticipated that it might act as an alkylating agent.

Since 5-halocytidine derivatives previously had been found to be more active antimetabolites than the corresponding uridine derivatives, it was of interest to prepared hydroxycytidine for comparison with 5-hydroxyuridine (Roberts and Visser, 1952b). Attempts to prepare 5-hydroxycytidine by the procedure used for the synthesis of 5-hydroxyuridine (Roberts and Visser, 1952a) were unsuccessful. A method involving interaction of a brominated intermediate on an anion-exchange resin produced the desired product. Elemental analysis, color tests and spectrophotometric data were used to establish the identity of the products.

Experimental

5-(p-Chlorobenzylidene)-aminouridine·Hydrate.—A solution of *p*-chlorobenzaldehyde, 0.54 g (0.0038 mole) in 25 ml of hot ethanol, was heated for 5 minutes with 15 ml aqueous aminouridine, 1 g (0.0039 mole). Upon concentration of the solution, crude crystals of the product, 1.22 g (80%), were obtained. Repeated recrystallization from hot absolute ethanol yielded white crystals which sintered at 137°, resolidified at 155°, and melted at 227–8° (uncorr.); $\lambda_{max}^{pH\ 7}$ 261 mμ; $\lambda_{min}^{pH\ 7}$ 228 mμ in phosphate buffer.

Anal. Calcd. for $C_{16}H_{16}O_6N_3Cl\cdot H_2O$ (399.80): C, 48.07; H, 4.54; N, 10.51. Found: C, 48.04; H, 4.79; N, 10.88.

5-(2',4'-Dichlorobenzylidene)-aminouridine·Hydrate.—A procedure similar to that described for preparation of 5-(*p*-chlorobenzylidene)-aminouridine was used. Aminouridine, 1 g (0.0039 mole), was reacted with 2,4-dichlorobenzaldehyde, 0.68 g (0.0039 mole). The crude product, 1.6 g (90%), which formed upon standing, was recrystallized repeatedly from absolute ethanol to give crystals which sintered at 133–34°, resolidified at 155°, and melted at 232-33° (uncorr.); $\lambda_{max}^{pH\ 7}$ 264 mμ; $\lambda_{min}^{pH\ 7}$ 235 mμ in phosphate buffer.

Anal. Calcd. for $C_{16}H_{15}O_6N_3Cl_2\cdot H_2O$ (434.25); C, 44.25; H, 3.95; N, 9.68. Found: C, 44.14; H, 4.27; N, 9.89.

5-(2',4'-Dichlorobenzylidene)-aminodeoxyuridine·Hydrate.—Aminodeoxyuridine hydrochloride, 1.5 g (0.0054 mole), was dissolved in 30 ml of water and the *p*H adjusted to 4 with sodium hydroxide. 2,4-Dichlorobenzaldehyde, 0.96 g (0.0055 mole) dissolved in 75 ml hot ethanol, was added to this solution. The reaction mixture was maintained at 70° for about 5 minutes and allowed to cool. Crystals of the crude product, 1.5 g (67%), were recrystallized repeatedly from hot absolute ethanol. The white crystalline product melted at 217° (uncorr.); $\lambda_{max}^{pH\ 7}$ 265 mμ; $\lambda_{min}^{pH\ 7}$ 237 mμ in phosphate buffer.

Anal. Calcd. for $C_{16}H_{15}O_5N_3Cl_2\cdot H_2O$ (418.25): C, 45.95; H, 4.10; N, 10.05. Found: C, 45.82; H, 4.30; N, 9.60.

5-(3',4'-Dichlorobenzylidene)-aminouridine·Hydrate.—The procedure described for the preparation of 5-(2',4'-dichlorobenzylidene)-aminouridine was followed. Crude product, 1.69 g (100%) from 1 g (0.0039 mole) aminouridine, was recrystallized repeatedly from absolute ethanol to yield white crystals, m.p. 149–150° (uncorr.); $\lambda_{max}^{pH\ 7}$ 260 mμ; $\lambda_{min}^{pH\ 7}$ 232 mμ in phosphate buffer.

Anal. Calcd. for $C_{16}H_{15}O_6N_3Cl_2\cdot H_2O$ (434.25): C, 44.25; H, 3.95; N, 9.68. Found: C, 44.50; H, 4.34; N, 9.78.

5-(3',4'-Dichlorobenzylidene)-aminodeoxyuridine·Hydrate.—The procedure described for the preparation of 5-(2',4'-dichlorobenzylidene)-aminodeoxyuridine was followed. Crude product, 1.71 g (76%) from 1.5 g (0.0063 mole) aminodeoxyuridine hydrochloride, was recrystallized from hot absolute ethanol to yield crystals which sintered at 135° and melted at 217° (uncorr.); $\lambda_{max}^{pH\ 7}$ 260 mμ; $\lambda_{min}^{pH\ 7}$ 233 mμ in phosphate buffer.

Anal. Calcd. for $C_{16}H_{15}O_5N_3Cl_2\cdot H_2O$ (418.25): C, 45.95; H, 4.10; N, 10.05. Found: C, 46.04; H, 4.19; N, 10.06.

5-Bromoacetamidouracil.—Aminouracil, 2 g (0.016 mole), was heated for 45 minutes to 1 hour under reflux with bromoacetylbromide, 20 ml. Two ml triethylamine was added slowly during the reaction period. The dark precipitate which formed upon standing was filtered. Crude product, 3.0 g (75%), was filtered off, washed with ethanol, and dried. Decolorization with norite and repeated crystallization from 70% ethanol yielded white crystals, m.p. 283–84° (with dec.); $\lambda_{max}^{pH\ 7}$ 251 mμ; $\lambda_{min}^{pH\ 7}$ 230 mμ in phosphate buffer.

Anal. Calcd. for $C_6H_6O_3N_3Br$ (248.05): C, 29.05; H, 2.44; N, 16.94. Found: C, 28.95; H, 2.53; N, 17.01.

5-Hydroxycytidine·Hydrate.—Bromine water was added to cytidine, 3 g (0.0123 mole), until a yellow color persisted for about 10 minutes. Excess bromine was removed by aeration, and the solution was passed slowly through a column (⅞ in. diameter) containing 100 ml IRA-4B (OH^-) resin. The effluent wash, which gave a blue color with a ferric chloride reagent (Heyroth and Loofbourow, 1934), was collected and lyophilized. The light amber-colored residue was crystallized from methanol-water to yield white crystals, 1.69 g (49.5%). Recrystallization from 95% ethanol gave crystals which turned opaque and melted at 242° (uncorr.); $\lambda_{max}^{pH\ 7}$ 292 mμ; $\lambda_{min}^{pH\ 7}$ 261 mμ in distilled water. The hydrochloride decomposed at 189°.

Anal. Calcd. for $C_9H_{13}N_3O_6\cdot H_2O$ (277.24): C, 38.99; H, 5.45; N, 15.16. Found: C, 39.32; H, 5.44; N, 15.43.

Spectrophotometric Determinations.—The λ of ultraviolet absorption maxima and minima were determined with the Beckman Spectrophotom-

TABLE I
ULTRAVIOLET ABSORPTION OF HYDROXY-SUBSTITUTED PYRIMIDINE DERIVATIVES

Compound	λ_{max} (mμ)	$\Delta\lambda_{max}$[a]	E_{max}	λ_{min} (mμ)	$\Delta\lambda_{min}$[a]	pH	Reference
Uracil (2,4-dihydroxy-pyrimidine)	260	—	8,200	228	—	7	National Research Council (1960); Pabst Laboratories (1955)
2,4,6-Trihydroxy-pyrimidine	255	5	24,500	224[b]	4	7	Heyroth and Loofbourow (1934)
2,4,5-Trihydroxy-pyrimidine	280	20	6,450	243	15	7	Heyroth and Loofbourow (1934)
Uridine[c]	262	—	8,800	230	—	—	Roberts and Visser (1952a)
Uridine	262	—	10,100	230	—	7	National Research Council (1960); Pabst Laboratories (1955)
5-Hydroxyuridine[c]	280	18	8,200	246	16	—	Roberts and Visser (1952a)
Cytidine[c]	270	—	8,830	247	—	—	Roberts and Visser (1952a)
Cytidine	271	—	8,900	249	—	7	National Research Council (1960); Pabst Laboratories (1955)
5-Hydroxycytidine[c]	292	22	7,837	261	11	—	—

[a] The extent of shift in ultraviolet absorption maxima and minima produced by substitution at the 5 or 6 position. [b] Approximate values. [c] Compound was dissolved in distilled water.

eter, Model DU. Each compound was dissolved in 0.067 M phosphate buffer (Hawk *et al.*, 1947) adjusted to *p*H 7.

Microbiological Methods.—Wild-type Neurospora and *E. coli* K-12 were test organisms used to screen the new compounds for their inhibitory effects on growth. Neurospora was grown for 2 days at 25° in 10-ml Erlenmeyer flasks containing 4 ml synthetic media and the test compound as previously described (Roberts and Visser, 1952b).

E. coli was grown for 18 hours at 37° in 5 ml of synthetic media containing varying amounts of the pyrimidine derivatives as described previously (Beltz and Visser, 1957). Growth in the presence of the inhibitors is expressed as percent of control growth based on turbidity measurements with the Klett-Summerson photometer (Filter No. 66).

The test compounds were dissolved in sterile media by warming to a maximum of 70°. These solutions were added to sterile media and inoculated directly. Degradation of the haloaryl Schiff's bases at these conditions was negligible. Decomposition products were not detected by descending paper chromatography with isoamyl alcohol saturated with water as solvent, whereas the probable degradation products, the 5-amino nucleosides and the aldehydes, were readily separated.

RESULTS AND DISCUSSION

Bromoacetamidouracil liberates bromide ion after being shaken for 5 minutes in 3 N NaOH at room temperature, whereas the bromine in 5-bromouracil is not labile under these conditions. The bromine in bromoacetamidouracil may be removed readily by warming in a fluorescein peroxide–acetic acid mixture (Feigl, 1946) to form dibromofluorescein. 5-Bromouracil liberates bromide with this reagent only after boiling the mixture to dryness.

The ion-exchange reaction used for the preparation of 5-hydroxycytidine is not applicable to the synthesis of 5-hydroxyuridine. When brominated uridine was passed through a column of IRA-4B (OH^-) resin, only a trace of material giving a blue color with ferric chloride was obtained. Although the product was not positively identified, it is assumed to be a 5,6-dihydropyrimidine derivative because of the similarity of its ultraviolet absorption spectrum to that of 5,6-dihydrouracil (Heyroth and Loofbourow, 1934). 5-Bromocytidine is the major contaminant of the mixture of compounds produced during the preparation of 5-hydroxycytidine.

Evidence that the hydroxyl substituent is at the 5 position of cytidine is provided by analysis of the spectrophotometric data. Substitution of a hydroxyl group at the 6 position of uracil produces a slight shift (Heyroth and Loofbourow, 1934; Cavalieri and Bendich, 1950) in λ_{max} (Table I). The introduction of a hydroxyl group at the 5 position of uracil or uridine produces a shift in λ_{max} (Roberts and Visser, 1952b: Cavalieri and Bendich, 1950) of 20λ and 18λ respectively. Since a similar bathochromic effect is noted upon introduction of a hydroxyl group into cytidine (see Table I), it is concluded that hydroxycytidine is probably substituted at the 5 position. Supportive evidence that the new compound is a 5-hydroxy derivative is provided by the fact that hydroxycytidine produces a blue color with ferric chloride (Lythgoe, 1948; Shugar and Fox, 1952).

Inhibitory effects of the new derivatives on growth of wild type Neurospora and *E. coli* K-12 are summarized in Table II. The data include inhibitory effects of the unsubstituted 5-amino derivatives and the aldehydes used to prepare the Schiff's base. All the new compounds completely inhibit growth of *E. coli* K-12 at different minimal concentration levels. In Neurospora, all the Schiff's base compounds are more effective inhibitors than the corresponding free amines. Nucleo-

TABLE II

INHIBITORY EFFECTS OF PYRIMIDINE DERIVATIVES ON GROWTH OF *E. coli* K-12[a] AND NEUROSPORA[b]

Pyrimidine Derivatives	Neurospora (μmole/ml[c])	*E. coli* K-12 (μmole/ml[c])
5-*p*-(Chlorobenzylidene)-aminouridine (Ia)	1.25 (50%)[d]	0.0375
5-(2′,4′-Dichlorobenzylidene)-aminouridine (IIa)	0.52	0.064
5-(2′,4′-Dichlorobenzylidine)-aminodeoxyuridine (IIb)	0.46	0.012
5-(3′,4′-Dichlorobenzylidene)-aminouridine (IIIa)	0.17	0.151
5-(3′,4′-Dichlorobenzylidene)-aminodeoxyuridine (IIIb)	0.30	0.012
5-Bromoacetamidouracil (IV)	2.16 N.I.[e]	0.020
5-Hydroxycytidine (V)	1.80 N.I.[e]	0.361
5-Aminouracil	3.93 N.I.[e]	0.191 N.I.[e]
5-Aminouridine	1.93[f]	0.13
5-Aminodeoxyuridine	1.77[g] N.I.[e]	0.012[g]
p-Chlorobenzaldehyde	0.571	—
2,4-Dichlorobenzaldehyde	0.229	0.229
3,4-Dichlorobenzaldehyde	0.115	0.286 N.I.[e]

[a] Incubated at 37° for 18 hours in glucose-salts medium. [b] Grown for 48 hours at 25° in synthetic media. [c] Minimal concentration of inhibitory compounds required to produce complete inhibition. [d] Fifty per cent growth, the maximum inhibition attainable. [e] N.I., no inhibition. [f] M. Roberts, Ph.D. Thesis, University of Southern California, 1951. [g] R. E. Beltz, Ph.D. Thesis, University of Southern California, 1956.

sides, amino acids, or vitamins (Table III) did not reverse the effects of any of the inhibitory compounds in Neurospora. 5-Hydroxycytidine or 5-bromoacetamidouracil inhibit growth of *E. coli* completely, but are without effect in Neurospora.

The aldehydes used to prepare the Schiff's bases are toxic at relatively low concentration levels (Table II). However, the inhibitory effects of the Schiff's base compounds are not the result of decomposition to free aldehyde and the amine. This conclusion is based on chemical stability tests and the data of Tables III, IV, and V, which show that Schiff's bases of aminouridine and aminodeoxyuridine prepared from the same aldehyde are markedly dissimilar in their biological activity. Furthermore, the reversing agents which are effective in the presence of the Schiff's bases do not relieve the inhibition produced by minimal concentration levels of the free aldehydes.

Various compounds were tested for their effectiveness as reversing agents in the presence of minimal concentrations of the dichlorobenzylidine derivatives which inhibit growth of *E. coli* completely. Tables III, IV, and V summarize these data.

TABLE III

REVERSAL OF *E. coli* K-12 AND NEUROSPORA GROWTH BY AMINO ACIDS AND CERTAIN VITAMINS IN THE PRESENCE OF 5-(2′,4′-DICHLOROBENZYLIDENE)-AMINOURIDINE[a]

Addenda	μmole/ml[b]	% Maximum Growth
E. coli		
Glutathione	0.014	100
L-Threonine	0.06	60–85
DL-Serine	0.06	50
Glycine	0.06	40–50
DL-Alanine	0.03	40
L-Cysteine·HCl	0.0006	20
L-Cystine	0.0006	20
L-Methionine	0.0006	20
L-Tryptophan	0.0006	20
L-Lysine	0.06	20
DL-Leucine	0.03	20
DL-Histidine	0.014	20
Folic acid	0.005	20
Pyridoxine·HCl	0.0024	20
Pyridoxal	0.0024	20
Inactive compounds[c]	—	0
Neurospora[d]		
Uridine	0.52	20–50
Cytidine	0.52	20–50
Deoxyuridine	0.52	20–50
Thymidine	0.52	20–50
L-Methionine	0.52	20–50
DL-Serine	0.52	20–50
Glycine	0.52	20–50
Folic acid	0.0013	20–50
Inactive compounds[e]	—	0

[a] Minimum concentration, 0.064 μmole/ml, inhibitor which suppressed growth completely during an 18-hour incubation period at 37° in glucose-salt medium. [b] Minimum concentration of reversing agent which promotes growth to the maximum attainable with the single reversing agent indicated. [c] Aspartate, L-glutamate, L-glutamine, L-phenylalanine, L-tyrosine, L-valine, uridine, cytidine, deoxyuridine, and thymidine gave no response over a wide range of concentration levels. [d] Concentration of inhibitor, 0.52 μmole/ml, producing complete inhibition of growth for 2 days at 25° in synthetic medium. [e] Glutathione, cysteine·HCl, cystine, and threonine produced no response at the concentrations tested.

In *E. coli*, the inhibitory effects of the aryl Schiff's bases prepared from aminouridine (IIa and IIIa in Table II) are reversed completely with glutathione (Tables III, IV). Cysteine, cystine, or histidine relieve completely the inhibitory effect of IIIa, but these amino acids are only partially effective as reversing agents in the presence of IIa. Single additions of several other amino acids or folic acid partially relieve the inhibitory effect of IIa or IIIa. Pyridoxine or

TABLE IV

REVERSAL OF *E. coli* K-12 GROWTH BY AMINO ACIDS AND CERTAIN VITAMINS IN THE PRESENCE OF 5-(3′,4′-DICHLOROBENZYLIDINE)-AMINOURIDINE[a]

Addenda	μmole/ml[b]	% Maximum Growth
E. coli		
Glutathione	0.015	100
L-Cysteine·HCl	0.015	100
L-Cystine	0.006	100
DL-Histidine	0.012	100
L-Methionine	0.075	88
Glycine	0.3	80
L-Threonine	0.077	70
L-Tryptophan	0.151	30
Inactive compounds[c]	—	0

[a] Inhibitor concentration 0.151 μmole/ml. This concentration suppressed growth completely during the 18-hour incubation period. [b] Minimum concentration of reversing agent to produce maximal growth stimulation. Higher concentration did not increase maximal effect. [c] DL-Alanine, DL-leucine, DL-serine, pyridoxine·HCl, pyridoxal, folic acid, uridine, cytidine, deoxyuridine, and thymidine gave no response over wide concentration levels.

TABLE V

REVERSAL OF *E. coli* K-12 GROWTH IN THE PRESENCE OF INHIBITORY CONCENTRATIONS OF 5-(2′,4′-DICHLOROBENZYLIDENE)-AMINODEOXYURIDINE[a] OR 5-(3′,4′-DICHLOROBENZYLIDENE)-AMINODEOXYURIDINE[a]

Addenda	μmole/ml[b]	% Maximum Growth
E. coli		
Uridine	0.004	100
Cytidine	0.004	100
Deoxyuridine	0.004	100
Deoxycytidine	0.004	100
Thymidine	0.004	100
Methionine	0.0001	20
Inactive compounds[c]	—	0

[a] Concentration of inhibitor, 0.012 μmole/ml, which suppresses growth completely at 37° for an 18-hour incubation period in glucose-salts medium. [b] Minimum concentrations of reversing agent to produce maximal growth response. Higher concentrations did not increase maximal growth. [c] Glutathione, cysteine·HCl, cystine, serine, glycine, histidine, threonine, alanine, folic acid, pyridoxine·HCl, and pyridoxal were ineffective.

pyridoxal are partially effective in the presence of IIa but have no effect in the presence of IIIa.

Inhibitory effects on growth of *E. coli* produced by the Schiff's bases prepared from aminodeoxyuridine (IIb, IIIb) are reversed completely by uridine, deoxycytidine, deoxyuridine, or thymidine (Table V). Methionine reverses the inhibitory effect to a maximum of 20% of control growth. None of the other amino acids is effective. IIb (0.46 μmole/ml) or IIIb (0.30 μmole/ml) completely inhibits mycelial growth of Neurospora for 2 days. These inhibitory effects are not reversed by the sulfur-containing amino acids or by serine, glycine, threonine, folic acid, pyridoxine, the pyrimidine ribonucleosides, or deoxyribonucleosides.

It is apparent that amino acid metabolism, rather than nucleic acid metabolism, is involved in the inhibitory effects of the aminouridine Schiff's bases in *E. coli* (Tables III, IV). On the other hand, the fact that nucleosides reverse the inhibitory effects of the corresponding aminodeoxyuridine derivatives indicates that these antimetabolites interfere with nucleic acid metabolism in *E. coli*. More data are required to determine the specific nature of these inhibitory processes.

The activity of bromoacetamidouracil in *E. coli* was relieved completely by glutathione and partially by cysteine. No other agent tested was effective. Bromoacetamidouracil was injected intraperitoneally in single doses of 2 or 3 mg into Swiss mice bearing the Ehrlich ascites tumor at 5 days after transplantation. It was found that the compound caused severe damage to tumor cells.[1] Within 1 to 2 hours after treatment the tumor cells began to show disintegration, stickiness, and clumping of chromosomes. Cells with pycnotic nuclei began to appear, and the damage increased progressively for at least 24 hours.

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Preparation and Properties of Beef Pancreas Microsomal Fraction*

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The microsomal fraction from beef pancreas homogenates has been prepared. It contains almost four times as much protein as RNA and about 25% phospholipid. It is most stable in solutions of low ionic strength at *p*H 7.0–7.4. Ribonucleoprotein particles of 80 *S* sedimentation rate can be separated from the membranous component by adjustment to *p*H 8.0. These particles are also detached from the membranous material by the detergents deoxycholate and lubrol. Chelating agents, on the other hand, dissociate the nucleic acid from the protein, with the latter forming aggregates of high molecular weight.

Current interest in the mechanism of protein biosynthesis has directed attention to the properties of the endoplasmic reticulum (microsomal fraction) and the ribonucleoprotein particles (ribosomes) of cells. Electron micrographs suggest that tissues which produce proteins primarily for secretion possess an extensive reticulum with attached particles, whereas tissues which synthesize proteins for utilization inside the cell of manufacture possess many unattached ribosomes (Harris, 1961). The pancreas, as an example of the former type, has been shown to synthesize RNase[1] in the microsomal fraction in the mouse (Morris and Dickman, 1960) and to incorporate labeled amino acid into chymotrypsinogen of guinea pig ribosomes (Siekevitz and Palade, 1960). To determine whether the attached ribonucleoprotein particles are the sole site of synthesis in a particular tissue, however, requires knowledge of their separation from the other components of the microsomal fraction. Results of such a study on beef pancreas microsomal fractions are included in this report. These data serve as a basis for the subfractionation procedures utilized in the accompanying paper (Dickman *et al.*, 1962), in which the incorporation of C^{14} amino acids into the proteins RNase, trypsinogen, and chymotrypsinogen A has been investigated in beef pancreas slices.

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[1] The abbreviations used are EDTA, ethylenediaminetetraacetic acid; PP_i, inorganic pyrophosphate; RNase, ribonuclease; RNA, ribonucleic acid.

EXPERIMENTAL

Homogenization and Fractionation.—Pancreas, preferably from Holstein cattle, was obtained about 10 minutes after slaughter and immediately immersed in ice-cold 0.25 M sucrose. All subsequent operations were carried out at 4°. Gross fat and connective tissue were removed, the glandular tissue forced through a stainless steel screen (1-mm holes), and the resulting pulp mixed with 9 volumes of chilled solution and homogenized with 4 strokes in a Potter-Elvejhem type homogenizer equipped with a Teflon pestle.

Differential centrifugation was accomplished with a Spinco Model L centrifuge. A large granule fraction (cell debris, nuclei, zymogen granules, mitochondria) was separated at 26,000 $\times$ *g* for 10 minutes and the microsomal fraction at 90,000 $\times$ *g* for 40 minutes. Each pellet was washed once by resuspension and recentrifugation.

Treatment of Microsomal Fraction.—When the microsomal fraction was to be further treated with detergents or by *p*H adjustment, the washed pellet was usually resuspended in mM phosphate by gentle homogenization. One-ninth volume of the detergent was added and the chilled suspension adjusted to the desired *p*H with the aid of a Leeds and Northrup *p*H meter equipped with miniature