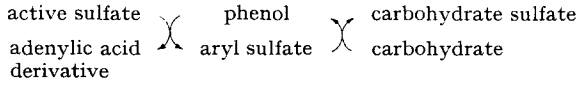
(measured at infinite thickness) of the radioactive sulfur was transferred from *p*-nitrophenyl sulfate to charonin sulfate, while no such reaction was observed at zero time nor when the *p*-nitrophenyl ^{35}S-sulfate in the system was replaced by ^{35}S-K_2SO_4.

The following considerations indicate that the other radioactive substance with an R_F value of 0.19 is glucose monosulfate: (1) 1.5 mg synthetic glucose-6-monosulfate was dissolved in 0.03 ml of the test solution and chromatographed on paper as described above. Glucose monosulfate appeared as a brown band on heating at 105° C for 5 min after spraying with an aniline hydrogen phthalate reagent. It was observed that the autoradiographic tracing overlapped with this visualisation. (2) If the substance is a reducing glucose monosulfate, it must be converted by benzylamine into the corresponding N-benzyl glycosylamine, and thus a considerable increase in R_F value may be effected as demonstrated by BAYLY AND BOURNE[4]. After 0.03 ml of the test solution was put on the paper, 0.035 ml of a 4% solution of benzylamine in methanol was superposed. The paper was heated at 85° C for 5 min, and then the same solvent as described above was allowed to ascend. The autoradiograph thus obtained is shown in Fig. 2. As can be seen, a considerable increase in R_F value (about 2.5 fold) was obtained.

From these experiments, it was concluded that ^{35}S-sulfate was incorporated into carbohydrates from *p*-nitrophenyl ^{35}S-sulfate by enzymic transsulfation and no incorporation took place from inorganic ^{35}S-sulfate. Further work is now in progress on the purification of the enzyme participating in the transsulfation and on the isolation of natural aryl sulfates in the mucous gland. However, it should be noted here that the transsulfation was inhibited by phosphate and fluoride in the same way as the aryl sulfate hydrolysis by arylsulfatases. This seems to suggest the participation of arylsulfatase itself in the reaction.

In the studies on the conjugation of sulfate with phenols, it was demonstrated by several workers that the primary step of the conjugation is an enzymic activation of inorganic sulfate by ATP. Recently HILZ AND LIPMANN[5] and DE MEIO AND WIZERKANIUK[6] have found that the active sulfate is an adenylic acid derivative. Their finding and the present evidence invite a tentative formulation of the sulfate transfer:



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Factors altering the activities of ribonuclease A and ribonuclease B

The specific question which led to the present investigations was that of the relation between the specificity of the major ribonuclease components (ribonuclease A and ribonuclease B) in the breakdown of ribonucleic acid (RNA).

In earlier investigations[1,2], we showed that ribonuclease B liberated 9.8 to 13.8% of guanylic acid, while crystalline ribonuclease, which contained both components A and B, liberated only 0.9 to 1.2%. Furthermore some synthetic activities of crystalline ribonuclease observed by HEPPEL, WHITFELD AND MARKHAM[3,4], were demonstrated[2] to occur with ribonuclease B.

The action of crystalline ribonuclease appears complex in nature, and depends on the proportion of the 2 fractions, A and B, acting together. The action of ribonuclease A and ribonuclease B, alone and combined in various proportions, has been investigated. The results reported here

indicate that the specific actions of RNase A and RNase B are altered when the 2 fractions are combined *de novo*.

Crystalline ribonuclease "Armour" was chromatographed on a column of carboxylic acid ion-exchange resin, Amberlite IRC-50, as described by HIRS, STEIN AND MOORE[5], to obtain a major peak designated RNase A and a minor peak, designated RNase B. Both peaks were re-chromatographed and each fraction crystallized from alcohol[6]. Yeast ribonucleic acid was prepared as reported previously[7].

Enzymic hydrolysis was carried out by mixing 0.5 ml of phosphate buffer containing 10 mg RNA with 0.25 ml of a solution of 0.2 mg of each of the RNase samples in 0.2*M* phosphate buffer, both at pH 7.2 and incubated at 37° C for 48 hours. 10 μl chloroform were used as a bacteriostatic agent.

Chromatograms of the digests of RNA with each of the RNase A, RNase B, RNase A and RNase B in 1:1 ratio and in 1:0.2 ratio were developed by the descending technique in two dimensions. The solvent systems used were (1) *iso*butyric acid–NH_3–H_2O (66:1:33)[8] buffered to pH 3.6, and (2) *iso*propyl alcohol–acetic acid–H_2O (6:3:1)[9]. Permanent records of the chromatograms were obtained by the photographic technique[10]. The located mononucleotides areas were cut from the chromatograms, and eluted by 5 ml 0.01*N* HCl. The ultraviolet absorption spectrum for each mononucleotide was determined by the use of a Beckman model DU spectrophotometer and the concentration of each calculated. Blanks were eluates of similar filter papers cut from blank chromatograms.

The differences in the activity observed in Table I depend on the quantitative relationships between the two components, and vary with the sequence of application of the enzymic fractions. The action of RNase B in liberating guanylic acid was either modified or inhibited by the presence of RNase A, depending on the relative proportion of the 2 enzymic fractions. The altered actions of RNase B by the action of RNase A, or of RNase A by the action of RNase B may be due to competitive inhibition resulting from enzyme-substrate complex formation[11] by each of the two ribonuclease components.

TABLE I

MONONUCLEOTIDES LIBERATED FROM YEAST RIBONUCLEIC ACID BY RIBONUCLEASE A AND RIBONUCLEASE B

	Uridylic acid moles per 100 moles	*Cytidylic acid* moles per 100 moles	*Guanylic acid* moles per 100 moles	*Adenylic acid* moles per 100 moles
RNase A (alone)	18.6	16.7	1.0	0.0
RNase B (alone)	13.0	12.2	13.8	1.3
RNase A–RNase B (1:1)	16.5	13.2	7.2	3.5
RNase A–RNase B (1:0.2)	18.2	15.7	1.4	3.7
1st RNase A, then RNase B (1:1)*	7.5	10.5	8.5	3.8
1st RNase B, then RNase A (1:1)*	18.2	15.7	14.9	3.7

* The first enzyme was incubated with the substrate for 24 h, then the second enzyme was added in equivalent amount, and incubation was allowed to proceed for further 24 h.

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