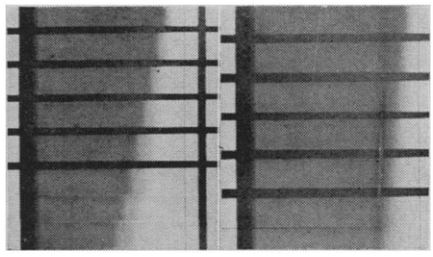

Fig. 3. Analytical ultracentrifuge pattern of RNA from ribonuclease treated and control 30*S* and 50*S* ribosomes. Ribosomes, suspended in tris, KCl, Mg (10^{-4}) buffer, *p*H 7.8, were treated with 5 μg of ribonuclease per milliliter. Control ribosomes treated as in Fig. 2. Rotor speed 39,460 rev/min; first picture taken 9 min after rotor attained speed; interval between pictures, 4 min; solvent 0.1*M* NaCl plus 0.01*M* acetate, *p*H 4.6, and RNA concentration 15 μg/ml. Left, two-component system of 23*S* and 16*S* RNA. Right, RNA from ribonuclease treated ribosomes.

ysis of the RNA by ribonuclease. Ribosomes in the 30*S* and 50*S* form were therefore incubated with ribonuclease for the same time period that 70S and 100S ribosomes were treated. The purified RNA was examined in the model E ultracentrifuge and the results of this experiment are shown in Fig. 3. It is obvious that even more extensive

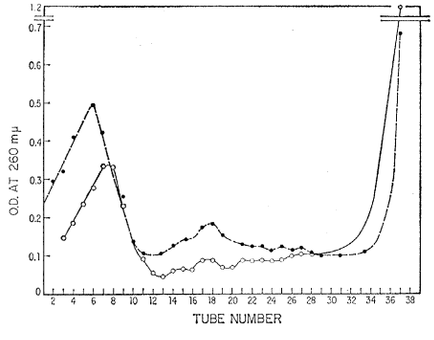

Fig. 4. Sucrose-gradient analysis of ribonuclease treated and control 30*S* and 50*S* ribosomes. Extract of *E. coli* was prepared in a 0.01*M* tris buffer at *p*H 7.8 containing 0.02*M* KCl 10^{-4}*M* Mg acetate and 1 μg deoxyribonuclease (*7*) per ml. Ribosomes were collected and resuspended in the same buffer and incubated at room temperature for 40 min with ribonuclease at a concentration of 5 μg/ml. At the end of the incubation period 0.1 ml portions were placed on top of a sucrose linear gradient (5- to 20-percent) prepared in the above buffer, and centrifuged in the SW39 rotor for 2.5 hours at 39,000 rev/min. Six drops per fraction were collected. To each fraction, 2 ml of water was added; the fractions were then read at 260 mμ. The bottom of the tube is at the left. Open circles, optical density (OD) values for ribonuclease treated 30*S* and 50*S* ribosomes. Solid circles, OD values for untreated ribosomes.

degradation of the ribosomal-contained RNA has taken place; no 23*S* or 16*S* RNA has remained. The 30*S* and 50*S* ribosomes which had been treated with ribonuclease were centrifuged on a 5 to 20 percent sucrose linear gradient in the SW39 swinging-bucket rotor to determine if extensive degradation of the ribosomes had taken place (Fig. 4). The ribonuclease-treated 50*S* and 30*S* ribosomes sediment in essentially the same position as control ribosomes, however, the total ultraviolet absorbing material is significantly reduced in the 50*S* and 30*S* components. Most of this ultraviolet-absorbing material appears where messenger RNA molecules would appear, toward the top of the sucrose gradient, and the rest is recovered at the top of the gradient probably as polynucleotides and mono- and dinucleotides of small molecular weight.

When ribonuclease is added to ribosomes under conditions of high magnesium ion concentration, the following sequence of events is suggested by the results of this study and the known action of ribonuclease. Ribonuclease attacks the 3′, 5′ phosphodiester linkages of those portions of ribosomal-contained RNA which are on the "surface" of the ribosome. At the moment, it is impossible to know how far the hydrolysis of the ribosomal RNA proceeds. It seems clear in any case that all 23*S* and 16*S* molecules are hydrolyzed to some extent. Under conditions where the 30*S* and 50*S* ribosomes are not associated, it appears that more RNA becomes accessible to ribonuclease attack. The point of attachment of 30*S* and 50*S* ribosomes may be a surface rich in RNA components or the structure of the ribosomes is altered by the lower magnesium concentration.

An analogous situation may be inferred from the experiments of Darnell reported by Jacob (*10*). Cells of *E. coli* which were heavily labeled with P^{32} and allowed to undergo extensive radioactive decay were still active in supporting phage growth, indicating that the structure of the ribosome, as a 70*S* monomer, was maintained in spite of breaks in the RNA chains. Phenol extraction of ribosomes which had undergone radioactive phosphorus decay did not yield 23*S* and 16*S* RNA.

The suggestion that RNA appears as a surface component in ribosomes raises the question of its functional significance in this position. It may be that the ribosomal RNA participates in the attachment of messenger RNA to the ribosome which would require having ribosomal RNA as a surface component. In addition, the association of 30*S* and 50*S* ribosomes may depend partially on their surface RNA components.

Although it appears from the data cited by Jacob that the primary structure of the RNA is not necessary for ribosome function, the integrity of the ribosome must nevertheless be preserved for protein synthesis. This may be due to the necessity of maintaining the appropriate surface configuration of those sections of ribosomal RNA which function in protein synthesis (*10*; *11*).

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Reductive Dechlorination of DDT to DDD by Yeast

Abstract. *Labeled DDD [1,1-dichloro-2,2-bis(p-chlorophenyl)-ethane] was formed from C^{14}-labeled DDT in the presence of yeast. The formation of DDD from DDE [1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene] was not observed, indicating that a reductive dechlorination of DDT occurs.*

Commercial yeast cakes (*1*) were used in an investigation of the action of yeast on DDT (Fig. 1) that was labeled with C^{14} in the phenyl group. In most experiments, 1 g of yeast cake was added to an autoclaved medium consisting of 1 liter of water, 90 g of sucrose, 1 g of K_2HPO_4, and 2 g of $(NH_4)_2SO_4$. The *p*H of this solution was 7.05. About 8 μg of DDT, with

a radioactivity of 200,000 count/min, was added in 1 ml of acetone. In one experiment, half this amount of C^{14}-labeled DDE was added. This material was prepared by the alkaline dehydrochlorination of labeled DDT with MgO. Boiled yeast was used in a control experiment.

After incubation at about 25°C the cells were harvested, washed, ground, and extracted with acetone. The extracted material was taken up in *n*-hexane and cleaned with sulfuric acid prior to analysis by paper chromatography. In most of the analyses the trimethylpentane system and $AgNO_3$ spray reagent described by Mitchell (*2*) were used. The average R_F values obtained with this method are: DDD, 0.35; DDT, 0.59; and DDE, 0.70. Minute quantities of DDD, DDT, and DDE were applied to each chromatogram. After development and spraying, ultraviolet light was used to produce visible color spots. Radioactivity in 0.5-cm segments of the strips was determined in a liquid scintillation counter. Extracts of the cell-free media were also prepared and their radioactivity was measured. The results of these experiments are presented in Table 1.

Three extracts of cell-free media were analyzed chromatographically without the sulfuric acid treatment. In experiment No. 2, after 50 hours, 54 percent of the radioactivity in the extract was distributed as DDD, 32 percent as DDT, and 12 percent was distributed between the origin and the DDD. The corresponding figures after 187 hours were 71 percent as DDD, 7 percent as DDT, and 20 percent as slow-moving material. These chromatograms exhibited streaking near the origin, which is usually indicative of inadequate cleaning of the extract. In experiment No. 3, the extract of cell-free medium contained essentially unchanged C^{14}-labeled DDE and no streaking occurred. Only one-half the usual amount of yeast and medium were used in this experiment in order to maintain a constant ratio of cells and chlorinated hydrocarbon. The smaller amount of material extracted may account for the absence of streaking and radioactivity near the origin. The slow-moving activity in the extracts of experiment No. 2 may have been due to labeled DDD trapped in contaminating material. The distribution of radioactivity in the cell extract of experiment No. 3 was almost identical to that of the original C^{14}-labeled DDE; the 7 percent of the counts located within the DDT spot represent a shoulder on the peak of DDE radioactivity.

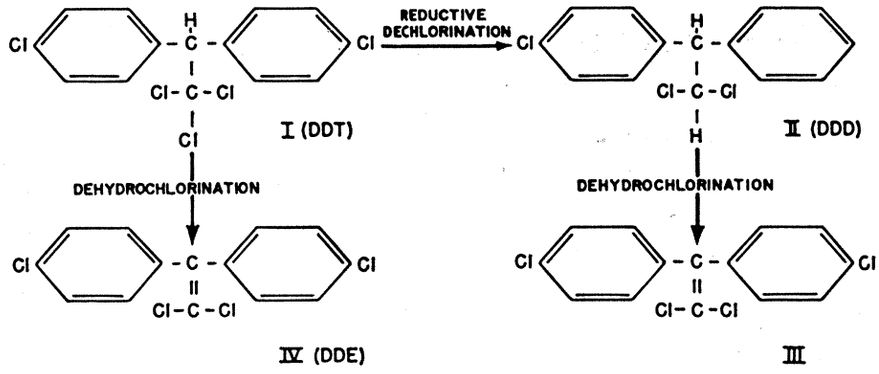

Fig. 1. The structure of DDT, and some related substances.

Table 1. Distribution of radioactivity after incubation of yeast cells with a medium containing C^{14}-labeled DDT or DDE.

Time (hr)	Final *p*H	Medium: added counts (%)	Cells: added counts (%)	Cell extract: percentage of counts on strip		
				With DDT	With DDD	With DDE
Experiment No. 1. C^{14}-labeled DDT, boiled yeast						
53	6.82	21	2	95	3	1
Experiment No. 2. C^{14}-labeled DDT, yeast						
40	4.10	8	10	58	40	1
50	3.69	5	18	25	73	1
72		6	21	11	88	1
187	3.33	5	26	7	85	1
Experiment No. 3. C^{14}-labeled DDE, yeast						
69	3.52	10	21	7	0	92

As further proof of the nature of the radioactive substance formed from labeled DDT, a chromatographic analysis of the extract was made with an aqueous solvent system, so that the order of migration of DDD, DDT, and DDE was reversed. In experiment No. 2, 83 percent of the radioactivity in the cell extract after 187 hours was distributed as DDD and 12 percent as DDT; there was no activity in the DDE area. Portions of the 40- and 72-hour cell extracts were pooled and passed through a column of MgO-celite. Chromatographic analysis of the material thus treated showed radioactivity throughout the DDE area, the area between DDE and DDT, and in the upper half of the DDT spot. None was present in the DDD area. This distribution indicates the presence of C^{14}-labeled DDE, formed by alkaline dehydrochlorination of DDT and of the labeled dehydrochlorinated derivative (III in Fig. 1) of DDD.

About 75 percent of the radioactivity added to each culture was not recovered. Insignificant levels of radioactivity remained in the solvent-extracted medium and in the acetone powder of the yeast. Various solvent washes of the flasks removed little additional activity, and studies with aqueous suspensions of C^{14}-labeled DDT at the same concentration used in the yeast cultures indicated that little or no adsorption on glass occurred. Since the amount of unrecovered activity was similar with boiled and living yeast, it appears probable that a physical process such as codistillation was responsible.

These data show that yeast has the ability to form DDD from DDT by reductive dechlorination, and that the reaction does not require the intermediate formation of DDE. A relatively simple method is thus provided for preparing labeled DDD and its dehydrochlorinated product from labeled DDT.

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3 July 1963