Breakdown of Rat Liver Polyribosomes by Benzene[1] (34852)

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During the course of exploratory tests regarding binding *in vivo* of labeled hydrocortisone (1,3-^{3}H-hydrocortisone in benzene–methanol, 10%) to rat liver ribosomes, disaggregation of polyribosomes was observed on sucrose-density gradients. Additional experiments showed that the observed polysome breakdown after the intraperitoneal administration of tritiated hydrocortisone to rats was caused by benzene. These observations led me to further explore the effect(s) of the intraperitoneal injection of benzene on polysome structure and *in vitro* protein synthetic activity.

Materials and Methods. Wistar strain male rats (Hilltop Lab Animals, Inc., Scottsdale, Pa.) weighing approximately 250 g were injected intraperitoneally with 3.37 mmoles of benzene (Fisher) per 100 g body weight and were sacrificed by decapitation after approximately 20 min. Rat liver cytoplasmic ribosomes were prepared according to the method of Wettstein *et al.* (1). In brief, the postmitochondrial supernatant fraction obtained after centrifugation at 36,000*g* for 30 min was adjusted to 0.5% with sodium deoxycholate and ribosomes were prepared by centrifugation through a layer of 0.5 *M* sucrose over 2 *M* sucrose at 150,000*g* for 2.5 hr. The pelleted material was used as a source of mono- and polyribosomes.

Polyribosome patterns were resolved on 7–34% logarithmic sucrose-density gradients containing 5 m*M* Tris–HCl, pH 7.6, 70 m*M* KCl, 4 m*M* Mg^{++} acetate, and 6 m*M* mercaptoethanol. The absorbance at 260 mμ was continuously registered with a Gilford 2000 recorder by forcing the gradient upward at a constant rate (40 ml/hr) through a specially designed fractionator connected to a Gilford flow cell (2).

Results and Discussion. Figure 1 shows the sedimentation pattern of polyribosomes prepared from postmitochondrial liver supernatant fractions of untreated and benzene-treated animals. Treatment with benzene *in vivo* for 20 min caused disaggregation of polysomes with concurrent appearance of an abnormally high concentration of ribosome peaks sedimenting in the monomer–dimer region of the gradient.

Figure 2 shows a time-course graph of L-^{3}H-phenylalanine incorporation into protein with ribosome and pH 5.1 fractions from normal and benzene-treated animals. Endogenous L-^{3}H-phenylalanine incorporation with fractions from benzene-treated animals is approximately 50% less than that obtained with fractions from normal animals. The possibility that benzene affects the pH 5.1 enzyme fraction was ruled out by hybrid experiments in which ribosomes from either benzene-treated or untreated animals were matched with pH 5.1 enzyme fractions from either source. pH 5.1 fractions from benzene-treated animals were as effective as pH 5.1 preparations from untreated animals in *in vitro* protein synthetic tests.

Breakdown of liver polysomes with a monomer–dimer accumulation was reported upon administration to rats of actinomycin D (3, 6), puromycin (4, 6), and carbon tetrachloride (5, 6). The occurrence of large amounts of ribosomal monomer–dimers was also found in neoplastic tissues (2, 7).

Polyribosome breakdown after treatment with toxic agents is not completely understood (4). However, since liver polysomes,

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[2] OD = optical density; cpm = counts per minute; mRNA = messenger RNA.

after treatment with benzene *in vivo,* become disaggregated and significantly less active in *in vitro* protein synthetic experiments, it seems reasonable to deduce that the effects of benzene are brought about via disorganization of the endoplasmic reticulum in a manner

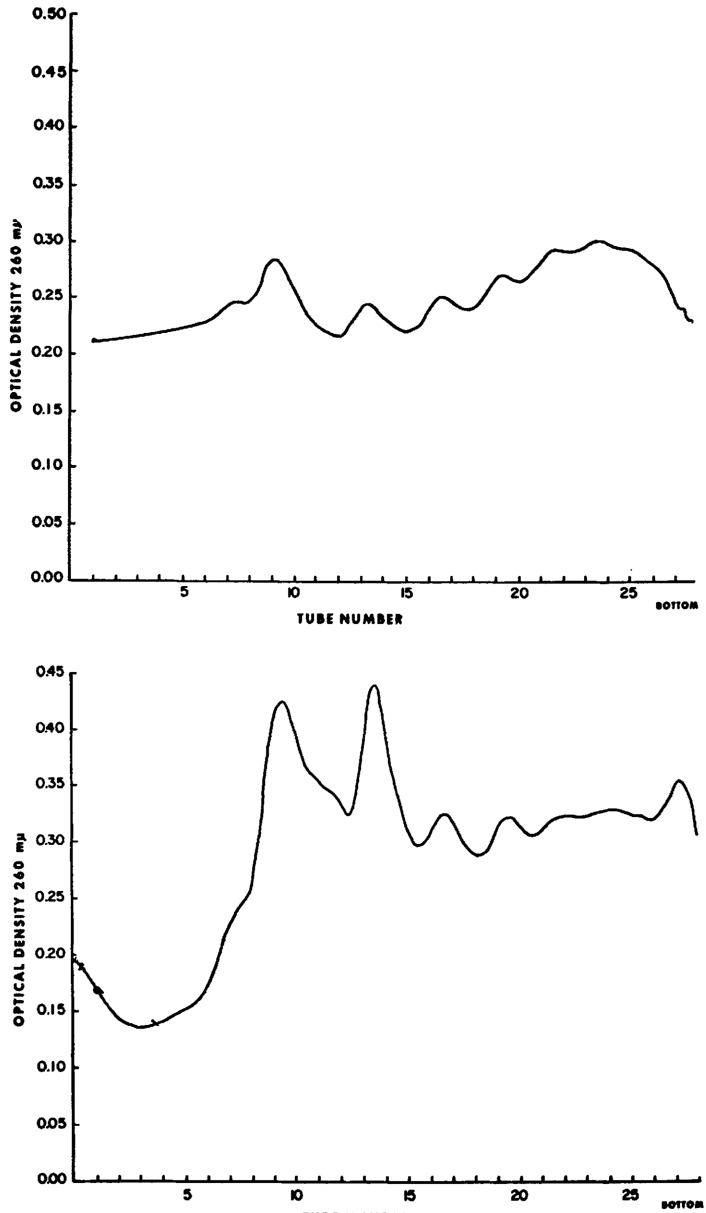

FIG. 1. Sucrose-density sedimentation patterns of liver polysomes of normal and benzene-treated animals. Polysomes were prepared from rat liver as described by Wettstein *et al.* (1) before and after the intraperitoneal administration of benzene. The preparations were resolved on 7–34% logarithmic sucrose-density gradients, as described previously (2). The absorbance at 260 mμ was continuously recorded with a Gilford 2000 recorder. The results were repeated 15 times with different ribosomal preparations. Subcellular preparations were prepared by combining the liver supernatant fractions from two or three animals. The figure shows representative data. Top. Control run (untreated). Bottom. After treatment with benzene *in vivo*.

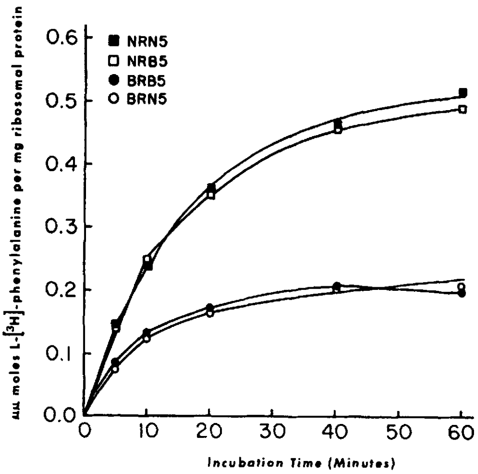

FIG. 2. Time-course of L-^{3}H-phenylalanine incorporation *in vitro* with ribosome and pH 5.1 fractions from untreated and benzene-treated animals. The graph is a typical *in vitro* protein-synthesis experiment. Incubation was carried at 37° as described (9) and, in this case, 1 mg ribosomal protein, 2 mg pH 5.1 enzyme protein, and 0.4 μCi L-^{3}H-phenylalanine (sp act 1 Ci/mmole, Schwarz Bio-Research, Inc.) were included in 0.6 ml of incorporation system. Aliquots of 0.1 ml were removed during incubation and protein was extracted by the Mans and Novelli (10) procedure. Radioactivity was measured using Bray's solution (11), as described (9, 2). Protein was measured by the method of Lowry *et al.* (12), and pH 5.1 enzyme was prepared by the procedure of Von Der Decken and Campbell (13). Each curve in the figure was repeated with at least four but not more than six different subcellular preparations. The highest variation (range) in these repeat tests was ± 0.08 μμmoles of L-^{3}H-phenylalanine per mg ribosomal protein. Protein synthesis tests were always run in duplicate with practically the same results. The figure shows representative results: NRN5 = ribosomes + pH 5.1 enzyme from normal animals. BRB5 = ribosomes + pH 5.1 enzyme from benzene-treated animals; NRB5 = ribosomes from normal animals + pH 5.1 enzyme from benzene-treated animals; BRN5 = ribosomes from benzene-treated animals + pH 5.1 enzyme from normal animals.

similar to that thought to occur in CCl_4 poisoning (5, 6, 8). On the other hand, it is quite conceivable that benzene treatment could damage cellular structure in such a way as to liberate ribonuclease activity which in turn acts on mRNA[2] causing polysome disaggregation and decrease in endogenous ribosomal protein synthetic function.

Summary. Administration of benzene to rats intraperitoneally causes breakdown of liver polyribosomes and appearance of a high concentration of particles sedimenting in the monomer–dimer region of the gradient. Ribosomes from benzene-treated animals were functional in *in vitro* protein synthesis but their endogenous capacity to incorporate L-^{3}H-phenylalanine into protein was significantly less than that of the controls (50%). There was, however, no difference in the pH 5.1 enzyme activities of benzene-treated and untreated animals.

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