Also when b4 RNA (6.7 mg) was first treated with ribonuclease (*5*), the number of plaques was reduced to the background level of 0 to 8.

To ascertain whether the lymphoid cells of the injected rabbits also produced IgG of allelic allotype, the whole serum, the isolated and concentrated IgG of the serum, and the lysed lymphoid cells were assayed by double diffusion in agarose gel with anti-b4 and anti-b5. Thus, in the experiment shown in Fig. 1 where b4 RNA was injected into a b5 rabbit, the lymphoid cell lysates (lymph node and spleen cells) as well as the isolated and concentrated IgG (30 mg/ml) from the serum precipitated not only with anti-b5 but also with anti-b4 (Fig. 2). However, in the intact whole serum, b4 IgG was only present in sufficient quantity to cause a slight bend in the adjacent precipitin lines (Fig. 2). As expected, control experiments with the whole serum and the isolated and concentrated IgG (30 mg/ml) before the rabbit was injected with b4 RNA failed to reveal the presence of the allelic allotype in the b^5b^5 homozygous rabbit.

The RNA conversion of lymphoid cells to synthesize IgM and IgG of foreign, allelic light chain allotype is qualitatively in accord with the data obtained for the RNA conversion of lymphoid cells in vitro (*5–7*); however, the magnitude of the response was less. This is readily explained by the dilution of the RNA in vivo and possibly also by the partial degradation of the intact RNA by ribonuclease of body fluids prior to its contact with lymphoid cells. The amounts of RNA which are maximally effective and the length of time that its effect will last remain to be determined.

In our previous in vitro study we could not detect anti-SRBC antibody activity in our intact RNA extracts (or after digestion with ribonuclease) using the direct or enhanced indirect hemolysis-in-gel technique; quantitative considerations excluded the possibility that the appearance of light chains with foreign allotype might be due to the passive transfer of IgG or IgM antibody molecules as contaminants of the RNA extracts (*6*). Similarly, in our experiments in vivo, the passive transfer of antibody molecules as an explanation of our results seems to be ruled out since the RNA extracts injected into the rabbits also had no detectable anti-SRBC activity. Furthermore, immunoglobulin light chains could not be detected in our RNA extracts by double diffusion in agar gel (Fig. 2) or by the quantitative inhibition of precipitation technique with anti-b4 or anti-b5 (*8*). Considering the fact that only about 5 mg of RNA in less than 1 ml of solution was injected into the rabbits, it is hardly conceivable that, after distribution via the circulation, sufficient antibody and immunoglobulin could have been "hidden" in the RNA extract and then be readily detected 18 hours later in the serum and lysates of spleen and lymph nodes.

Thus it appears that the RNA-mediated conversion of lymphoid cells is due to the transfer of information contained in the base sequence of RNA. One possibility is that the RNA acts as the direct template for protein synthesis. It is also intriguing to consider the possibility that the genetic information of foreign RNA is stabilized in the host cell by a de novo synthesis of DNA through the activity of an RNA-dependent DNA polymerase system, as found by Baltimore (*9*) and by Temin and Mizutani (*10*) for mammalian cells infected with RNA viruses. Nevertheless, the possibility must also be considered that the structural genes for the b4 and b5 polypeptide chains are not truly allelic. Thus, in spite of the extensive genetic data available which is consistent with allelism (*11*), the DNA for the b4 and b5 polypeptide chains may indeed be present in both b4 and b5 homozygous rabbits and the allelic genes controlling the synthesis of these polypeptide chains may be control rather than structural genes and act by allowing either the b4 or b5 DNA chromosomal regions to be expressed. If this were so, the RNA extracts might be modifying the action of the control genes. In either event, the observations reported here are directly pertinent to the molecular biology of antibody synthesis and cellular differentiation.

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Carcinogen and Microsomal Membrane Interactions: Changes in Membrane Density and Ability to Bind Nucleic Acids

Abstract. *DNA and synthetic copolymer polyribocytidylic-polyriboguanylic acid bind to microsomal membrane. The nucleic acid–membrane complex may be detected by centrifugation in cesium chloride density gradients. The density of the nucleic acid–membrane complex and, in certain cases, the amount of nucleic acid associated with the membrane was changed in the presence of various carcinogenic chemicals.*

The technique of equilibrium density gradient centrifugation in cesium salts has yielded valuable information on the properties and the metabolism of nucleic acids (*1*). During our studies on interactions between cellular membranes and nucleic acids (*2*) we used this method to detect binding of nucleic acids to purified membranes. Such complexes are formed spontaneously between various proteins and nucleic acids and can be detected by a variety of methods (*2, 3*). In CsCl, RNA and DNA have densities of 1.9 and 1.7

g/cm^3, respectively; the membrane bands at a density close to 1.2 g/cm^3. Thus, the detection of the added labeled nucleic acids with the membrane at this or some intermediate density is interpreted and defined as binding.

It became apparent early in our studies that of all the nucleic acids tested [DNA from *Escherichia coli* and various RNA fractions from *E. coli* and Ehrlich ascites cells; synthetic polyribonucleotides (*4*): poly A, poly C, poly G, poly U, and various combinations thereof] only DNA and the copolymer of poly C·poly G formed stable complexes with the microsomal membrane from rat liver. We now report on the influence of various chemical carcinogens on the density of microsomal membrane in CsCl and on the binding of two nucleic acids to this intracellular structure. The carcinogenic activity of the chemicals used in our study has been recently reviewed (*5*).

The microsomal membrane from rat liver (*6*) formed one visible band in CsCl at the density of 1.18 g/cm^3. If the membrane was first incubated with labeled poly C·poly G (Miles) before centrifugation in CsCl, the radioactivity was detected at this density in the gradient. However, radioactive *E. coli* DNA (*7*) was distributed in two peaks; one coincided with a visible band at density 1.18 g/cm^3, while the other was detected at a density of 1.22 g/cm^3 (Fig. 1). Data in Fig. 1 are actually a composite of two parallel experiments in which the membrane was incubated with the individual polymer. Traces of poly C·poly G strongly inhibited binding of DNA to the membrane.

When certain carcinogenic chemicals were present during the incubation of the nucleic acid with the membrane, the density of the latter and consequently of the membrane–nucleic acid complex was changed drastically (Fig. 1, B–F). Incubation with AAF (*8*) distributed the visible bulk of the membrane between several bands ranging in density from 1.16 to 1.22 g/cm^3. Most of the DNA was associated with the band 1.22 g/cm^3, while two minor DNA peaks localized at a density of 1.18 and 1.13 g/cm^3, poly C·poly G was found complexed to the membrane at a density of 1.15 and 1.17 g/cm^3 with a minor peak at 1.22 g/cm^3.

Incubation of the membrane with N-Ac-AAF (Fig. 1C) produced two bands when analyzed on the CsCl gradient, a white-colored zone with a density of 1.25 g/cm^3 and a yellow zone 1.19 g/cm^3. DNA was associated predominantly with the former, and poly C·poly G with the latter. The reason for the different colors of these two bands is not clear, but metabolic transformations of the carcinogen by the individual membrane fractions may be responsible.

The interactions of N-OH-AAF with the membrane (Fig. 1D) produced complexes of various densities in the range of 1.19 to 1.25 g/cm^3. The labeled nucleic acids were distributed between several of these bands, some of which had densities different from the visible membrane material.

While AAF and its derivatives caused a distinct fractionation of the membrane into two or more bands, incubation with either MTT or BPL (Fig. 1, E and F) resulted in a single visible band with a density lower than that of the native membrane. In the case of BPL, DNA and poly C·poly G were found associated with the membrane at 1.16 g/cm^3. Most of the material, however, which binds these macromolecules after incubation with MTT is found at densities greater (1.18 g/cm^3) than that of the visible bulk of the membrane material. It should be noted that the "ultimate" carcinogens (*5*) such as N-Ac-AAF and BPL have a distinct tendency to increase the amount of nucleic acid bound to the membrane. This is especially true for DNA incubated in the presence of BPL (Fig. 1E). The carcinogen-induced changes in the membrane density are reproducible from one experiment to another, although minor variations are occasionally observed between different batches of membrane material. The presence or the absence of the added nucleic acids (in the concentrations used) has no influence on

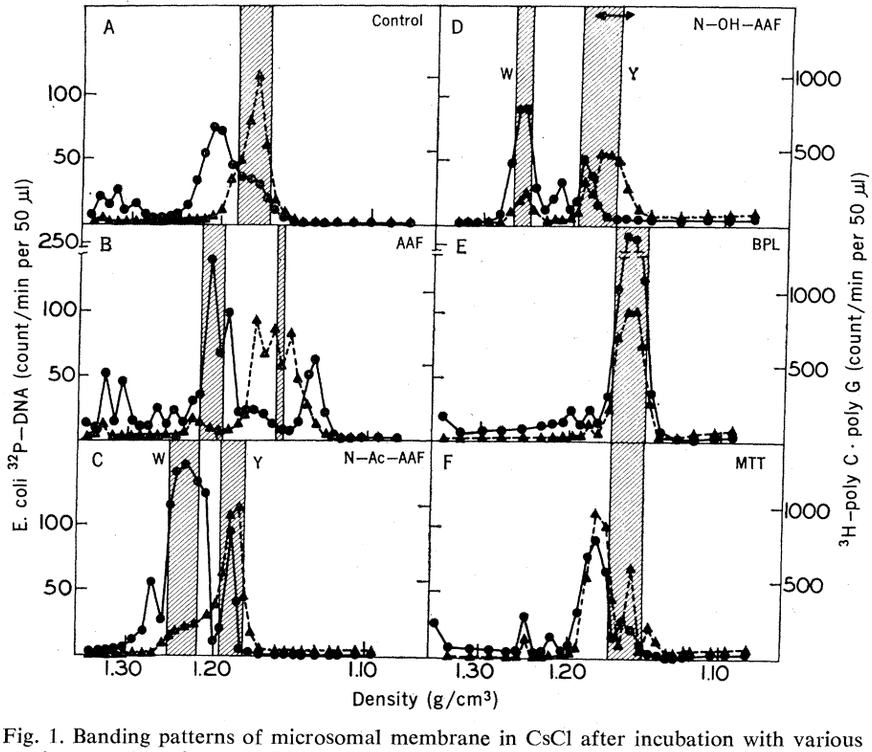

Fig. 1. Banding patterns of microsomal membrane in CsCl after incubation with various carcinogens. Membrane (500 µg of protein per sample) was incubated at 40°C in buffered salt solution (0.1*M* NaCl, 0.02*M* phosphate buffer, *p*H 7.2) with ^{32}P-labeled DNA from *E. coli* (5 µg) or [^{3}H]poly C·poly G (5 µg of each of the two homopolymers) in the presence and in the absence of the chemical carcinogens. The concentration of BPL was 4 mg/ml, and that of the other carcinogens was 2 mg/ml. After 60 minutes of incubation, the samples were diluted 13-fold with a buffered salt solution (0.14*M* NaCl, 10^{-3}*M* phosphate buffer *p*H 7.2). Saturated CsCl solution was added to the required density (refractive index = 1.352 ± 0.001); the samples were covered with mineral oil and centrifuged (Spinco SW 41 rotor) 38,000 rev/min for 30 hours. The content of each tube was collected from the bottom, and the location of the visible membrane material was determined and recorded. The refractive indices were established for several fractions, and the densities of these fractions were read from tables (*11*). Portions (50 µl) were withdrawn for measurements of ^{32}P or ^{3}H radioactivities. ▲——▲, [^{3}H]poly C·poly G; ●———●, [^{32}P]DNA *E. coli*. The double-headed arrow in rectangle D denotes the calculated position of the membrane band in the absence of any carcinogen (control). The shaded bars show the position of the visible membrane material; *W* and *Y* indicate white- and yellow-colored bands, respectively.

the banding patterns of the membrane-carcinogen complexes.

Our experiments show that (i) CsCl equilibrium density gradient centrifugation is useful for the study of membrane–nucleic acid and membrane-carcinogen interactions, (ii) the chemical carcinogens studied so far interact with the membrane to change its density and, in certain cases, fractionate the membrane into two or more distinct classes, (iii) both the degree and the pattern of binding of nucleic acids to microsomal membrane may be influenced by the carcinogen, and (iv) the changes described in (ii) and (iii) are different for each of the compounds studied.

It is not clear whether multiple membrane zones observed after incubation with AAF and its derivatives are due to certain existing differences within the liver microsomal membrane population or whether the membrane was modified to varying extents during the incubation with the carcinogen. The first possibility suggests that there exist in rat liver two or more classes of membranes each binding preferentially either a particular carcinogen or its metabolite to form a complex with a new density in CsCl. The second scheme suggests that all members of the population of membranes have equal chance to react with either the carcinogen or its hydrolysis or metabolic products. We favor the first scheme since it appears that preexisting receptors for the two nucleic acids are separated and distributed at new densities in the gradient in the presence of AAF and its derivatives. This is suggested by the quantitatively similar binding of the nucleic acids in the control experiment and in the presence of AAF and N-OH-AAF.

Several other substances such as antibiotics, pesticides, and metal ions also changed the density of the microsomal membrane in CsCl and altered the pattern of the nucleic acid binding. Each compound changes these patterns in its own specific and unique way, although, as found in other experiments, substances related chemically tend to have similar activities. The available data suggest that several biologically active molecules, including chemical carcinogens, act by interfering with the chemistry and the biological activity of cellular membranes. Binding of chemical carcinogens to endoplasmic reticulum was first reported more than a decade ago (*9*). Whether the observed reactions between the carcinogens and the membrane may be involved in the process of tumor induction or in the perpetuation of the malignant state (or both) is not clear. Although additional experiments are required to evaluate this possibility further, alterations of the cellular surfaces observed during malignant transformation (*10*) make this suggestion plausible.

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Euglossine Bees as Long-Distance Pollinators of Tropical Plants

Abstract. *Euglossine bees may return to a nest from as far away as 23 kilometers in a tropical rain forest. These bees apparently forage long distances and visit the same plants repeatedly along a feeding route. They probably promote outcrossing among tropical plants with low population density; therefore, they may permit the existence of plant species whose densities have been forced very low by such things as competition and predators on seeds and seedlings.*

Euglossine bees (*Euplusia, Eulaema, Euglossa*) are important pollinators of lowland neotropical woody plants (*1, 2*). There have been many studies of their nesting and social behavior (*2*) and on the obligatory mutualistic interaction between euglossine males and the orchids they pollinate (*3*). The spectacular pollination activities of the males are probably of much less importance to tropical community structure than are those of the females, whose foraging behavior is almost unknown. I here report some details of this foraging behavior in hopes of encouraging field ecologists to study these bees before they become extinct through human destruction of neotropical vegetation (*4*).

Three lines of indirect evidence strongly suggest that euglossine females fly a much longer distance from nest to host, and between hosts, than is generally expected of bees (*5*).

Female *Euplusia surinamensis* were captured while sleeping in their nests in lowland Costa Rican rain forest, marked by wing notching, and stored in individual containers until the following day. They were transported in a dark box and two bees were released at 1, 2, 3, 4, 5, and 6 km from their nests at about 11:15 a.m. on a clear day. By 3:00 p.m. all had returned and resumed nest construction. A year later, this experiment was repeated, but 12 bees were released between 6:36 a.m. and 6:58 a.m. at 14, 17, 20, and 23 km from the nest, three bees at each distance. From these distances, two, one, three, and one had returned by nightfall. The fastest bees were from 20 km (65 minutes) and 14 km (47 minutes). These two bees returned with full pollen loads but the other five appeared not to have foraged for nesting materials. Of the five that did not return, three were just beginning cell construction and therefore may have had little experience at foraging away from the nest site. The