the majority of that size which had lost phosphorylase (Fig. 1).

We conclude that: (i) Fast alpha motor neurons, the largest in the motor neuron pool, belong to the group that is rich in phosphorylase and poor in SDH. (ii) Slow alpha motor neurons, considered on the basis of indirect evidence to be somewhat smaller than the fast ones (*3, 8, 9*), are also mainly if not entirely of this histochemical type, although we cannot exclude that a few might be of the opposite type. (iii) Renshaw neurons and other interneurons, whose axons do not form part of the motor roots, are not included in the phosphorylase-rich group because no neurons in the anterior horn remained positive for phosphorylase after root section; apparently they are small neurons rich in SDH and poor in phosphorylase. (iv) The majority if not all of the gamma motor neurons, because they are thought to be less than 30 μm in diameter (*4–9*), appear to be rich in SDH and poor in phosphorylase (Table 1).

The similarity between alpha motor neurons innervating fast twitch muscle and those innervating slow twitch muscle in regard to phosphorylase and SDH histochemical reactions is in sharp contrast to the histochemical differences (*13*) of the muscle fibers of those muscles, wherein the fibers of fast twitch muscle are mainly rich in phosphorylase (and glycogen) and poor in SDH, whereas those of slow twitch muscle are rich in SDH and poor in phosphorylase (*14*). The functional properties of the two types of alpha motor neurons are not as clearly distinct from each other as are the functional properties of the muscle fibers they innervate (*2, 3*). However, the differences between functional properties of alpha motor neurons as a group compared with those of Renshaw neurons, other interneurons, and gamma motor neurons are prominent and parallel the histochemical properties. Renshaw neurons (*7, 15*), other interneurons (*7*), and gamma motor neurons (*4, 7, 8, 16*) are capable of higher firing frequencies (also perhaps fire relatively more often). They are richer in mitochondrial oxidative enzyme activity as marked by SDH activity. In being better equipped for mitochondrial oxidative enzyme activity they resemble type I (slow twitch, red) muscle fibers. Alpha motor neurons fire at lower frequencies (*3, 4, 7, 8, 15, 16*) (also perhaps less often) and are richer in phosphorylase activity and glycogen content. Thus all the alpha motor neurons are like type II (fast twitch, white) muscle fibers in being better equipped for anaerobic glycolysis.

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Expression of Allelic Immunoglobulin in Homozygous Rabbits Injected with RNA Extract

Abstract. *Ribonucleic acid extracts obtained from lymph nodes of immunized rabbits homozygous for the* b^4 *or* b^5 *allele of light chain immunoglobulin allotypes were injected intravenously into nonimmunized rabbits homozygous for the alternate allele. Approximately 30 percent of the plaque-forming cells in the spleen yielded plaques with immunoglobulin M antibody possessing the allotype of the RNA donor. The allotype of the RNA donor was also found in the IgG immunoglobulin of lymphoid cell lysates as well as in the IgG isolated from the serum. These results suggest that the injected RNA has an informational role in the in vivo synthesis of immunoglobulins by host lymphoid cells.*

An informational role of immunologically active RNA extracts was suggested by the experiments of Adler, Fishman, and Dray (*1*) in which it was shown that the IgM (*2*) antibody to T2 phage synthesized by RNA-treated homozygous lymphoid cells in culture possessed light chains with allelic allotype genetic markers (*3*) characteristic of the RNA donor. Using sheep red blood cells (SRBC) as the antigen and the localized hemolysis in gel technique (*4*) to assess IgM and IgG antibody formation (*4*) by single cells, we extended these observations and reported (*5, 6*) that RNA extracts of lymphoid cells from immunized homozygous rabbits, with the b^4 or b^5 light chain allele (*2*), convert spleen cells from nonimmunized homozygous rabbits, with the b^5 or b^4 allele, respectively, to produce antibody of foreign light chain allotype. When the RNA was extracted from immunized rabbits 5 days after a single intravenous injection of 4×10^8 SRBC, "direct" plaque-forming cells (PFC) were observed; these could be inhibited by 2-mercaptoethanol (2-ME) or goat antibody to IgM (goat anti-IgM) but not by goat antibody to the Fc fragment of IgG (goat anti–Fc-IgG) indicating that the antibody produced by the PFC were of the IgM class (*5*). When the RNA was extracted from immunized rabbits 18 to 24 days after the first of several intravenous injections of SRBC, "indirect" PFC were observed by developing the plaques with goat anti–Fc-IgG; the "indirect" PFC were not inhibited by 2-ME or by goat anti-IgM but were inhibited by excess goat anti–Fc-IgG, an indication that the antibodies in the plaques were of the IgG class (*6*). The RNA was extracted from lymphoid cells homozygous for one allele, b^4 or b^5, controlling light chain allotypes of immunoglobulin, whereas the spleen cells were homozygous for the other allele, b^5 or b^4 (*5, 6*). By use of antibodies to the b4 and b5 allotypes, the allotypes of the IgM or IgG antibody produced in the plaques were identified (*5, 6*). Practically all of the "direct" plaques and most of the "indirect" plaques of the converted spleen cells possessed IgM or IgG antibody, respectively, with the allotype characteristic of the donor of the "immune" RNA extract (*5, 6*). Moreover, the allotype of the RNA donor

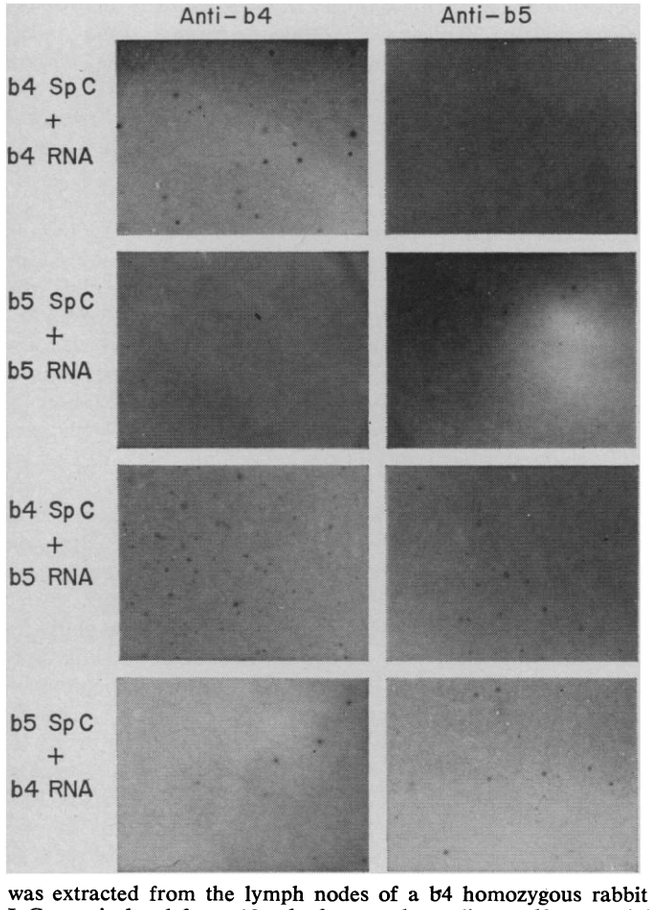
could be detected in the IgG of lysed spleen cells by immunodiffusion (*7*).

We now report the results of our experiments designed to determine whether RNA extracts of lymph nodes from a rabbit homozygous for b^4 or b^5 will convert the lymphoid cells of the host which is homozygous for the other allele, that is b^5 or b^4, to the in vivo synthesis of IgM and IgG of foreign light chain allotype. For this purpose, RNA was extracted from lymph node cells of rabbits 5 days after one intravenous injection of 4×10^8 SRBC. The RNA was injected intravenously into nonimmunized rabbits whose lymphoid tissues (mesenteric lymph nodes, popliteal lymph nodes, and spleen) were assayed 18 to 20 hours later (i) by the localized hemolysis-in-gel technique for plaques with IgM anti-SRBC antibodies of each allotype (*5*) and (ii) by immunodiffusion for IgG of each allotype. The techniques for RNA extraction and localized hemolysis in gel have been described (*5, 6*). The localized hemolysis-in-gel technique was modified (*5*) in that the plates were incubated for 4 hours instead of 1 hour before the addition of complement.

The allotype of the IgM antibody in the direct plaques was identified by radioautography with the use of [^{125}I]-IgG anti-b4 and [^{125}I]IgG anti-b5 (*5*) and also by inhibition of plaque formation with anti-b4 and anti-b5 antisera incorporated into the agarose (*5*). When b4 RNA (5.0 mg) was injected intravenously into b4 rabbits, labeled plaques were observed with anti-b4 but not with anti-b5 (Fig. 1); in separate plates, of 109 "direct" PFC per 10^6 spleen cells, 88 percent were inhibited by anti-b4 and 5 percent were inhibited by anti-b5. Similarly, when b5 RNA (5.0 mg) was injected into b5 rabbits, ^{125}I-labeled plaques were observed with anti-b5 but not with anti-b4 (Fig. 1); of 75 "direct" PFC per 10^6 spleen cells, 4 percent were inhibited by anti-b4 and 91 percent were inhibited by anti-b5.

On the other hand when b4 RNA (4.8 mg) was injected into a b5 rabbit or when b5 RNA (5.2 mg) was injected into a b4 rabbit, significant numbers of labeled plaques were observed when either anti-b4 or the anti-b5 was used (Fig. 1); of the 127 and 96 "direct" plaques observed, 30 and 43 percent were inhibited by anti-b4 and 45 and 32 percent were inhibited by anti-b5. Thus, 30 to 32 percent of the plaques possessed light chain allotypes of the RNA donor.

Essentially similar results were found for the antibody forming cells of popliteal and mesenteric lymph nodes, with RNA and spleen cells from other rabbits and with lymphoid cells obtained 5 days after the RNA injection. In control experiments, two rabbits receiving a single intravenous injection of 4×10^8 SRBC yielded only 26 and 37 plaques after 18 to 20 hours, a result consistent with those reported (*5, 6*).



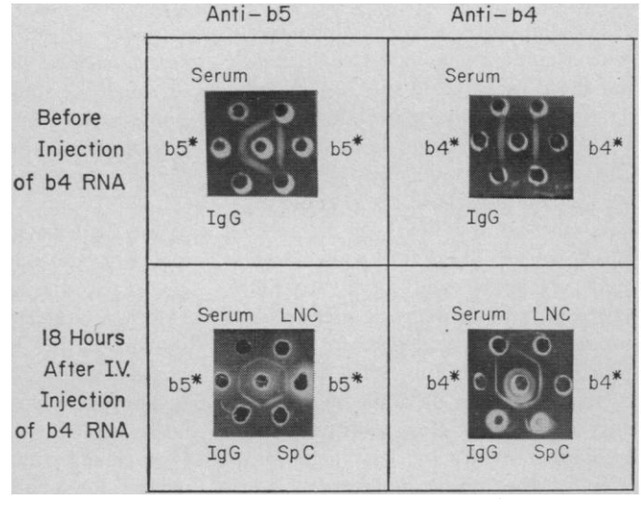


Fig. 1 (left). Identification of the light chain allotypic specificities of IgM antibodies in PFC of spleens taken 18 to 20 hours after rabbits were given a single intravenous injection of 4.8 to 5.2 mg of RNA in 1 ml of Hanks balanced salt solution. The RNA was extracted from lymph nodes of rabbits 5 days after a single injection of 4×10^8 sheep red blood cells. The allelic allotype of the RNA and spleen cell donor is indicated for each experiment. The b4 and b5 "direct" plaques were visualized by radioautography with ^{125}I-labeled IgG preparations isolated from anti-b4 and anti-b5 antisera (*5*). Fig. 2 (right). Identification of the light chain allotypic specificities of IgG immunoglobulin in whole serum, in isolated IgG preparations from serum, in lymph nodes cells (*LNC*) lysates, and in spleen cell (*SpC*) lysates obtained 18 hours after a b5 homozygous rabbit as given a single intravenous (*I.V.*) injection of 4.8 mg of RNA in 1 ml of Hanks balanced salt solution. The RNA was extracted from the lymph nodes of a b4 homozygous rabbit 5 days after a single injection of 4×10^8 sheep red blood cells. The IgG was isolated from 10 ml of serum by sodium sulfate precipitation, DEAE chromatography, and concentration by ultrafiltration so that the solution contained 30 mg of IgG per milliliter. The cell lysates were prepared from 5.4×10^9 spleen cells and from 6.2×10^9 lymph node cells in 1 ml of the Hanks solution; the cells were lysed by freezing and thawing and then concentrated to a volume of 0.2 ml. The allelic allotypes were identified by double diffusion in 1.5 percent agarose containing 0.75 percent deoxycholate in 0.1*M* sodium borate buffer at *p*H 8.6; anti-b5 and anti-b4 antisera were placed in the center wells, the reference b4* and b5* sera (from homozygous rabbits) were placed in the lateral walls. The serum and IgG in the upper patterns were obtained from the rabbit before the injection of RNA.

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