ment, are derived from the host. The fluorescent-antibody technique we used has an advantage over labeling with H^3-thymidine or chromosome marker techniques in that all cells of a given population, rather than just dividing cells, may be detected.

Our study is the first demonstration that these cells come from the bone marrow. Volkman and Gowans (*8*) have shown that phagocytic cells appearing at sites of nonspecific inflammation come directly from a dividing cell population in the bone marrow, and this finding was confirmed in our study by results obtained with turpentine. It is a reasonable inference that the same cell population participates in both specific and nonspecific reactions, and this hypothesis is supported by the fact that the percentages of marrow-derived cells found in each are similar. Thus the delayed reaction is comparable to the ameboid cell reaction in starfish larvae pricked with a rose thorn (*13*), differing only in the specificity of the trigger and the greatly increased intensity of the response.

The possibility that cells originating in the bone marrow sojourn in another organ before traveling to specific skin reaction sites appears to be ruled out by the discrepancy between their percentage in these sites and their percentage in peripheral lymphoid organs, the lymph nodes in particular. Our experiments exclude the thymus as a participant in the production or processing of these cells. Thus they are completely distinct from the population of specifically sensitized cells, which appear to originate in the marrow, pass through the thymus into the peripheral pool of immunocompetent cells, and undergo immunization in lymph nodes (*6*, *14*).

Our experiments, while they show that cells from the bone marrow can act as the source of cells participating in delayed skin reactions, do not establish unequivocally that they do so under normal physiological conditions. Conclusive proof of this relationship must be obtained in future investigations.

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15. The data presented here will be included in a thesis submitted to the Graduate School of Yale University in partial fulfillment of the degree of Doctor of Philosophy. Supported by NIH grants AI 06112 and AI 06455. One of us (D.M.L.) was supported by a PHS training grant (5 T1 AI291).

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Membranes in Polyribosome Formation by Rabbit Reticulocytes

Abstract. *When rabbit reticulocytes are incubated with* n-*butanol, an agent disruptive to the structure and function of cellular membranes, there is a rapid disaggregation of the polyribosomes. Reaggregation is promoted when the* n-*butanol is diluted below a critical concentration or when the cells are washed free of the alcohol and the incubation is continued. Neither disaggregation nor regeneration will occur in the absence of protein synthesis. These observations suggest that integrity of the reticulocyte membrane is necessary for the attachment of ribosomes to messenger RNA and for the formation of polyribosomes.*

Polyribosomes in rabbit reticulocytes disaggregate when the cells are incubated in the presence of tryptamine (*1*), homotryptamine, and α-ethyltryptamine (*2*). Baglioni and Colombo (*1*) indicated that tryptamine may act as other than an antagonist of tryptophan, and Hori *et al.* (*2*) suggested that the activity of these compounds may be related to some property of indoleamines. We have found that another heterocyclic amine, primaquine, causes disaggregation of polyribosomes in concentrations lower than those causing hemolysis. Primaquine induces changes in the permeability of erythrocyte membranes, resulting first in a loss of intracellular potassium and, in higher concentrations, in complete hemolysis (*3*). Therefore, it seems reasonable to assume that these various organic amines might cause polyribosome disaggregation by altering a cell membrane structure or functional component.

n-Butanol is another compound that induces changes in the permeability of erythrocyte membranes (*4*). At a concentration of 0.4 mole/liter, it causes a reversible loss of intracellular potassium and a gain in sodium ions (*5*) without inhibiting glycolysis (*6*). Since *n*-butanol is a dipolar and lipid-soluble molecule, it becomes concentrated in the lipid part of lipoprotein membranes of cells and brings about a disorientation of membrane structure and loss of function (*7*). *n*-Butanol appeared to be the agent of choice in an investigation of the role of membrane integrity on polyribosome formation, since its effects on cell function are considered to be a direct result of its action on membranes.

When rabbit reticulocytes were incubated in 0.1*M* *n*-butanol at 37°C, the polyribosomes disaggregated to monomeric ribosomes within 5 minutes (Fig. 1). No effect was observed if the concentration of the *n*-butanol was below 0.05 mole/liter. We prevented polyribosome disaggregation by inhibiting protein synthesis with cycloheximide (*8*) or by retarding the rate of protein synthesis by omitting the essential amino acid histidine (*2*, *9*). In the absence of *n*-butanol this concentration of cycloheximide or the omission of histidine had no effect on the ribosome-polyribosome profile. If the cells were treated with 0.1*M* *n*-butanol at 0°C no polyribosome disaggregation occurred; indeed, at this temperature a concentration of 0.4 mole/liter was ineffective. Polyribosomes were reformed either when the butanol was diluted with additional buffered medium or when the cells were washed with the medium and the incubation was continued at 37°C for 10 minutes (Fig. 1). This regeneration did not occur if protein synthesis was inhibited by cycloheximide, or if the cells were maintained at 0°C.

These results indicate that continued protein synthesis is necessary for both disaggregation and regeneration of the polyribosomes, and that *n*-butanol does not disrupt the ribosome–messenger RNA complex once it is formed. We hypothesize that the butanol acts on a membrane component responsible for the attachment of ribosomes to messenger RNA and for the formation of polyribosomes. Since the effect of *n*-butanol was observed before any apparent hemolysis occurred and since this compound was present in a concentration which did not elicit a loss of potassium or rubidium from the cells or a decrease in their ability to maintain adenosine triphosphate (ATP) (Table 1), formation of polyribosomes may be considered a specific and sensitive parameter of membrane function.

Recently, there has been considerable interest in the disaggregation of reticulocyte polyribosomes induced by fluoride ions (*10*). It has been suggested that fluoride acts by inhibiting a reaction responsible for initiation of the polypeptide chain (*11*). As in the case of *n*-butanol, the effect of fluoride is prevented when protein synthesis is inhibited by cycloheximide (*12*). Sodium fluoride affects permeability of the erythrocyte membrane (*13*) by a mechanism primarily due to a complexing of alkaline earth and fluoride with ligands in the cell membrane (*14*). Fluoride, in concentrations capable of affecting permeability of swine and human erythrocytes, lowers cellular concentrations of ATP only slightly (*15*), and it inhibits a membrane adenosine triphosphatase required in ion transport (*16*). Furthermore, the activity of this enzyme is decreased by organic solvents affecting the integrity of the membrane (*17*). Thus the action of fluoride and butanol appear to be similar, and it is likely that both of these agents cause disaggregation of polyribosomes by inhibiting a membrane component necessary for the formation of polyribosomes.

Table 1. Absence of effect of 0.1*M* butanol on the concentration of adenosine triphosphate (ATP) in and the release of ^{86}Rb by reticulocytes. The concentrations of adenosine triphosphate were determined on perchloric acid extracts of reticulocytes by the method of Kornberg (*22*). To estimate the effect of butanol on permeability, we first incubated whole blood (10 ml) from anemic rabbits with 20 μc of $^{86}RbCl$ (2.7 mc/mg) for 15 minutes. The cells contained approximately 8×10^4 count/min after they were washed twice with cold buffered saline. Incubation was performed as indicated, and samples of the incubation medium and of a lysate of the cells were counted in a gas-flow proportional counter with a Micromil window. Since the exchange rate of $^{86}Rb^+$ is virtually the same as that of K^+ (*23*), the results represent a sensitive estimate of potassium leakage from the reticulocytes. Determinations of potassium by flame photometry gave similar results. Incubations were performed for 5 minutes at 37°C in the absence (control) or presence (experimental) of *n*-butanol (0.1*M*). The values given are the means of five determinations ± the standard deviations.

Condition	ATP (μmole/0.25 ml of cells)	^{86}Rb released into medium (%)
Control	0.51 ± 0.08	5.7 ± 1.6
Experimental	.48 ± 0.07	6.5 ± 1.1

Polyribosomes disaggregate within the first few minutes of incubation in cell-free preparations capable of protein synthesis (*18*). This may be caused by their separation from a membrane component necessary for attachment of monomeric ribosomes to messenger RNA. From evidence obtained with bacteria (*19*) and mammalian liver cells (*20*), cytoplasmic membranes have been implicated as regulators of protein synthesis. Miller and Maunsbach (*21*) have presented electron-microscopic evidence that the primary determinant of hemoglobin synthesis in rabbit reticulocytes may be invaginations of the plasma membrane. Our results are in agreement with these findings and, furthermore, they point to membranes as requisites for a specific step, that of polyribosome formation and, as a result, for initiation of polypeptide chains. *n*-Butanol, by disrupting the lipoprotein configuration of the membrane, may weaken the essential bonds between the lipid and an initiation factor or enzyme that is an integral part of the membrane.

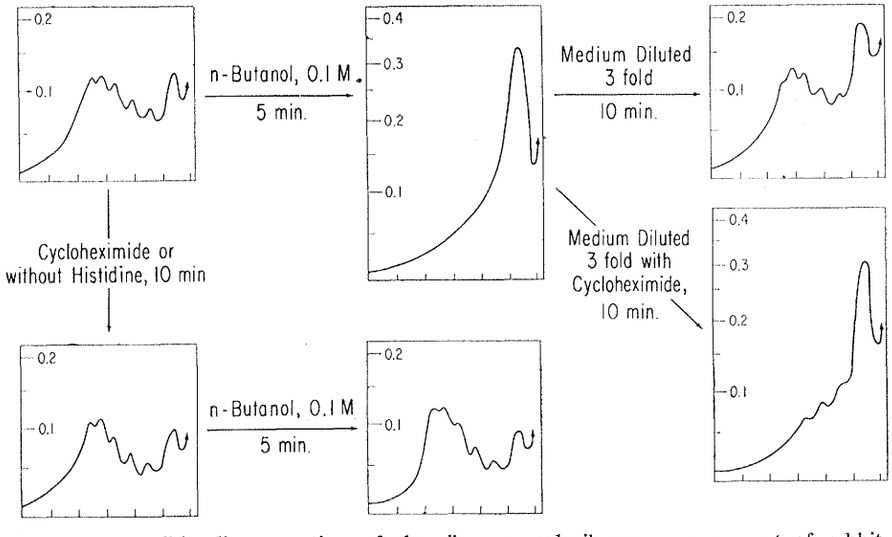

Fig. 1. Reversible disaggregation of the ribosome-polyribosome component of rabbit reticulocytes by *n*-butanol. Preparation of reticulocytes; incubation of the washed cells with glucose, amino acids, and buffered saline medium; lysis of the cells after incubation; zonal centrifugation in a sucrose density gradient; and measurement of the ultraviolet profile of the ribosome-polyribosome component have been previously described (*2*). The ordinates represent absorbancy at 260 mμ, and the abscissas represent the linear distance from the bottom of the 30-ml tube which is 6 cm long. The concentrations of *n*-butanol and cycloheximide were 0.1*M* and 0.01*M*, respectively. We initiated reaggregation of the polyribosomes by adding two volumes of buffered saline medium or by washing the cells with the medium and continuing the incubation for 10 minutes.

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Apomixis: Seasonal and Population Differences in a Grass

Abstract. *Changes in incidence of apomictic and sexual embryo sacs were detected in* Dichanthium aristatum *in an experimental population at six stations covering 27 degrees of south latitude, and during the flowering season in a wild population. Differences were associated with photoperiods prevailing during development of inflorescences. Response to length of day was quantitative, differing in the two strains.*

A cytological survey of inflorescences of *Dichanthium aristatum* grown under field conditions reveals striking changes in the incidence of apomixis during the flowering season. These changes are strongly associated with differences in photoperiod during development of inflorescences. Apomixis in *Dichanthium* is of the aposporous type (*1*), in which unreduced clonal embryo sacs may arise beside the reduced sexual megaspore. Such embryo sacs can be recognized cytologically, since a unique four-nucleate sac is produced (*2*), in contrast with the eight-nucleate sexual sac typical of the Gramineae. *Dichanthium*, a short-day plant, was selected because earlier experiments in controlled environments (*3*) had shown that the length of day at a critical phase during development of inflorescences controlled the incidence of apomictic embryo sacs.

I used two sources of material:

1) Strain CPI *14366*, a recent introduction from South Africa, is known to be a versatile apomict and is grown from seed at six stations differing in latitude along the eastern coast of Australia. The seed was obtained from plants grown in controlled environments under conditions promoting maximum apomixis (*3*).

2) Samples of approximately 12 inflorescences were collected at random at regular intervals from wild populations naturalized in northern Australia; all were at a similar stage of development, just preceding stigma exsertion. Inflorescences were immediately fixed in a mixture of ethanol and acetic acid (3 : 1 by volume) for 12 to 24 hours and stored in 70-percent ethanol. The cytological processing has been described (*3*), except that the material was embedded in Paraplast, and adhesion of sections to slides was facilitated by the amylopectin method of Steedman (*4*). The resultant data were based on analysis of at least 200 embryo sacs from four to six inflorescences for each sample. The embryo sacs were classified as sexual or apomictic according to earlier criteria (*3*). Approximately 5 to 10 percent of sacs, particularly those at a stage approaching maturation, could not be classified. Experiments in controlled environments have established that the minimum period for initiation and development of inflorescences is approximately 40 days (*5*), and this interval was used for extraction of the relevant climatic data.

Figure 1 shows the relative frequency of apomictic and sexual embryo sacs in inflorescences from a population of strain *14366* at stations between 9° and 36°S during first flowering in late summer—February 1964. Length of day during development of inflorescences strongly correlates with the incidence of apomixis. At the three southerly stations, where the day exceeded 14 hours throughout development of inflorescence, the incidence of apomictic sacs was low: 54.82 ± 3.46, 60.69 ± 3.72, and 63.08 ± 3.30 percent at stations *A*, *B*, and *C*, respectively. At the three northern stations, where photoperiods were less than 14 hours, it was high: 92.96 ± 1.41, 87.45 ± 2.01, and 91.40 ± 1.50 percent at stations *D*, *E*, and *F*, respectively. Other climatic factors, such as temperature, showed no clear association with the degree of apomixis.

Statistical analysis of these data indicates two quite distinct levels of apomixis, dependent on whether or not plants received photoperiods longer than 14 hours during floral development. Data for the three stations within each category were homogeneous [> 14 hours (*A,B,C*), $\chi^2 = 3.03$, with 2 degrees of freedom; < 14 hours (*D,E,F*), $\chi^2 = 5.54$, with 2 degrees of freedom], whereas the difference between the two categories was highly significant ($\chi^2 = 209.06$, with 1 degree of freedom). The mean percentage of apomictic embryo sacs for longer than 14 hours was 59.58 ± 2.03; for shorter than 14 hours, 90.84 ± 0.94. Geographically, this switch in the reproductive system occurred between latitudes 21° and 25°S, which is close to the latitude of origin of the race near Pretoria, South Africa (25°45′S).

The wild populations sampled were all tetraploid, some having a relatively constant high level (90 to 95 percent) of apomixis (see *6*). In other populations the degree of apomixis varied markedly during the flowering season. Figure 2 shows data from a ruderal population, *Mareeba-1*, from Mareeba, North Queensland (latitude, 17°00′S), where *D. aristatum* is known to have been naturalized for about 50 years. A sample collected in December, at the beginning of the flowering season in early summer, showed a low incidence of apomictic sacs (52.14 ± 4.22 percent), while a sample collected the following February (late summer) showed an increase to 78.09 ± 2.85 percent. Apomixis reached a maximum toward the end of the season, as judged by the April samples for 1963 and 1964: 90.71 ± 2.45 and 92.30 ± 1.98 percent, respectively.

The climatic data of Fig. 2 show that variation in photoperiod corresponds generally with patterns of temperature and rainfall, but unlike them it follows a precisely predictable course. Experiments in controlled environments (*3, 5*) have shown that apomixis in *D. aristatum* is controlled by the photoperiod during development of inflorescence; this evidence suggests that the seasonal variation in apomixis detected in *Mareeba-1* was controlled to a major extent by the lengths of day prevailing during floral development. The Decem-