the involvement of erythropoiesis in the "leukaemic degeneration"[7,8]. The decreased proliferative activity of the erythroblasts[9] may be a contributive cause of anaemia in this disease.

We thank Mrs Ursula Michlmayr for technical assistance. This work was supported by the fund "Kampf dem Krebs".

C. HUBER
H. HUBER
F. SCHMALZL
H. BRAUNSTEINER

Department of Medicine,
University of Innsbruck

Received April 24, 1970.

1 Killmann, S. A., *Ser. Haematol.*, **1/3**, 38 (1968).
2 Gavosto, F., Maraini, G., and Pileri, A., *Blood*, **16**, 1122 (1960).
3 Abbrederis, K., Schmalzl, F., and Braunsteiner, H., *Schweiz. Med. Wschr.*, **99**, 1425 (1969).
4 Huber, C., Lederer, B., Schmalzl, F., Bütterich, D., Huber, H., and Braunsteiner, H., *Acta Haematol.* (in the press).
5 Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R., and Robertson, J. S., in *The Kinetics of Cellular Proliferation* (edit. by Stohlman, F., jun.) (Grune and Stratton, New York, 1959).
6 Boll, I., and Ganssen, O., *Acta Haematol.*, **27**, 229 (1962).
7 Whang, J., Frei, E., III, Tjio, J. H., Carbone, P. P., and Brecher, G., *Blood*, **22**, 664 (1963).
8 Jensen, M. K., and Killmann, S. A., *Acta Med. Scand.*, **181**, 47 (1967).
9 Wintrobe, M. M., *Clinical Hematology*, sixth ed. (Lea and Febiger, Philadelphia, 1967).

Lipid Layer in Cell Membranes

MEASUREMENTS of the extent of shrinkage of membranes which results from hydrolysis of phospholipid components by phospholipase C provide a direct indication of the area occupied by lipid in cell membranes. Maximum treatment of erythrocyte ghosts with phospholipase C (*Clostridium welchii*) liberates approximately 70% of the membrane phospholipid phosphorus and the surface area of the ghosts is decreased by 45–55% as calculated from diameter measurements made on spherical ghosts observed by phase contrast microscopy[1] and more recently from ghost volume measurements by an exclusion technique involving the use of ^{14}C labelled sucrose. There is strong evidence that the phospholipid molecule as a whole is displaced from the membrane but the fate of the other membrane lipids is not established although it has been demonstrated that a residual lipoprotein structure persists. To indicate the quantitative significance of the observations, however, we have assumed that all lipids are removed in the same proportions as for the phospholipid. The observed shrinkage of the membrane by 50% would then be attributed to the loss of 70% of the membrane lipid. This would indicate that lipid occupies an area corresponding to $100 \times 50/70 = 70\%$ of the overall membrane area. Should any lipid be displaced to a lesser extent, then the figure for the area occupied by lipid would be increased to more than 70%.

An equivalent study[2] of muscle microsomes treated with phospholipase C demonstrated a shrinkage of 55% when 70% of the phospholipid of the membrane was hydrolysed. In this membrane phospholipid accounts for about 90% of the total lipid reported[3] so that the extent of phospholipid hydrolysis gives a much more accurate indication of the probable extent of displacement of lipid from the membrane. The conclusion drawn from these experiments is that lipid occupies at least 80% of the area of the muscle microsomal membranes.

Thus in two experiments involving a surface membrane and a membrane preparation derived mainly from endoplasmic reticulum (membranes with significantly differing lipid compositions) there is direct evidence that interruption of the lipid layer by other components such as protein amounts certainly to no more than 30% and probably much less. Consequently, because protein accounts for about 60% of the dried weight of the erythrocyte membrane preparation it can be calculated that at least two thirds of this protein must lie outside the lipid layer, perhaps to either side of it to form a sandwich structure of the type first suggested by Danielli and Davson[4].

Penetration of the lipid layer by proteins may still be a very important feature from the point of view of the stability of membrane structure and of membrane function, but it may be looked on as a refinement of the lipoprotein sandwich model. So too with the observation that the protein configuration approximates that of a globular protein and the suggested possibility that the lipid may to some extent and under certain conditions adopt a configuration other than that of a continuous bilayer. All of these details can be incorporated without difficulty in this kind of model which was always intended simply to represent an average picture of a membrane.

J. B. FINEAN
T. A. BRAMLEY
R. COLEMAN

Department of Biochemistry,
University of Birmingham,
Birmingham B15 2TT

Received August 6, 1970.

1 Coleman, R., Finean, J. B., Knutton, S., and Limbrick, A. R., *Biochim. Biophys. Acta*, **219**, 81 (1970).
2 Finean, J. B., and Martonosi, A., *Biochim. Biophys. Acta*, **98**, 547 (1965).
3 Martonosi, A., *Biochim. Biophys. Acta*, **150**, 694 (1968).
4 Danielli, J. F., and Davson, H., *J. Cell. Physiol.*, **5**, 495 (1935).

Competitive Hybridization with Brain RNA fails to confirm New RNA induced by Learning

MUCH circumstantial evidence implicates protein and RNA synthesis in memory consolidation and increasing interest in work directed toward elucidating the macromolecular chemical processes underlying learning[1,2]. A derepression model has been advocated by Bonner[3], who also suggested a method by which the model might be tested. Newly induced RNA molecules would be gene products, present in the brains of learning animals but not naive animals, and they should be able to be pulse labelled and detected by competitive hybridization experiments.

The "detection of RNA species unique to a behavioural task" by competitive hybridization has now been reported by Machlus and Gaito[4–6]. When 50 μg of DNA was hybridized first with 50 μg of unlabelled, naive brain RNA and then with 50 μg of labelled, learned brain RNA, there was about 50% competition. But when the DNA was hybridized first with unlabelled, learned RNA and then labelled, naive RNA, no radioactivity was observed; there was apparently 100% competition. This was taken as evidence that there were new species of RNA in the brains of the trained rats. Our data show no differences between the RNA from trained and naive rats. Control experiments and consideration of other data in the literature for hybridization with nucleic acids from higher organisms lead us to believe that the competition values reported by Machlus and Gaito are unrealistic and incorrect.

We have attempted to reproduce their results as follows: Sprague–Dawley male rats, 200–250 g, were injected intraventricularly with 40 μCi 5-^{3}H-uridine in physiological saline or with physiological saline. Naive rats were returned to their home cages and killed 90 min after injection. Trained rats were put into a training apparatus 60 min after injection and allowed to become accustomed to the box for 15 min, and then were given 15 min of shock avoidance training, fifteen trials in 15 min. They learned the task well within four or