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- Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquisee, M., Merrill, S. H., Penswick, J. R., and Zamir, A., *Science*, **147**, 1462 (1965).
- Zachau, H. G., Dutting, D., and Feldmann, H., *Physiol. Chem. Z.*, **347**, 212 (1966).
- Madison, J. T., Everett, G. A., and Kung, H., *Science*, **153**, 531 (1966).
- Baev, A. A., Venkstern, T. V., Mirzabekov, A. D., Krutilina, A. I., and Axelrod, V. D., *Mol. Biol.*, Moscow, **1**, 754 (1967).
- Rajbhandary, U. L., Stuart, A., Faulkner, R. D., Chang, S. H., and Khorana, H. G., *Proc. US Nat. Acad. Sci.*, **57**, 751 (1967).
- Dirheimer, G., Sabeur, G., and Ebel, J. P., *Biochim. Biophys. Acta*, **149**, 587 (1967).
- Dirheimer, G., and Ebel, J. P., *Bull. Soc. Chim. Biol.*, **49**, 1679 (1967).
- Gangloff, J., Keith, G., and Dirheimer, G., *Bull. Soc. Chim. Biol.*, **52**, 125 (1970).
- Madison, J. T., Everett, G. A., and Kung, H., *J. Biol. Chem.*, **246**, 1318 (1967).
- Uchida, T., Arima, T., and Egami, F., *J. Biochem.*, Japan, **67**, 91 (1970).
- Cramer, F., Doepner, R., Van De Haar, F., Schlimme, E., and Seidel, H., *Proc. US Nat. Acad. Sci.*, **61**, 1384 (1968).
- Levitt, M., *Nature*, **224**, 759 (1969).
- Crick, F. H. C., *J. Mol. Biol.*, **19**, 548 (1966).

Linkage Groups of the θ and Ly-A Loci

SEVERAL mouse alloantigens described recently are expressed solely or chiefly on lymphoid cells. This indicates that the programme of selective gene action responsible for cyto-differentiation includes instructions for the synthesis of unique cell surface conformations characteristic of particular cell types^{1,2}.

Among the various genetic loci responsible for these "differentiation antigens"¹ are *Tla* (ref. 1), θ (ref. 3) and *Ly-A* (ref. 4). Two special reasons for studying the linkage between them are first, that leukaemogenesis in the mouse is thought to be associated with genetic derepression at the locus *Tla*, and second, that in one case, genetic linkage (of *Tla* and *H-2*) is associated with the proximity of the gene products (TL and H-2 antigens) in the assembly of molecules which constitute the cell surface, a provocative isolated example of a relationship between the linkage of genes and the structural organization of their products.

In summary, the data obtained so far suggest that θ is in linkage group II at a distance of 16.8 ± 3.6 units from *d* (*dilute*) and that *Ly-A* is in group XII at a distance of 26.6 ± 4.2 units from *ru* (*ruby-eye*). The θ and *Ly-A* phenotypes were scored by

Table 1 Linkage Between θ and *d* (II)

	θ -AKR ⁺ +/d	θ -AKR ⁻ d/d	θ -AKR ⁺ d/d	θ -AKR ⁻ +/d	
(RF × DBA/2)♀ × DBA/2♂	35	40	5	6	86
DBA/2♀ × (RF × DBA/2)♂	8	6	6	1	21
	43	46	11	7	107

Antiserum for θ -AKR antigen: C3Hf/Bi anti-AKR thymocytes.

Table 2 Linkage Between *Ly-A* and *ru* (XII)

	Ly-A.1 ⁺ +/ru	Ly-A.1 ⁻ ru/ru	Ly-A.1 ⁺ ru/ru	Ly-A.1 ⁻ +/ru	
(C3H/An × C57Bl/6J-ru)♀ × C57Bl/6J-ru♂	39	44	16	14	113

Antiserum for Ly-A.1 antigen: (BALB/c × C57Bl/6) anti-C57Bl/6-Ly-A.1 (congenic strain) thymocytes; complementation with selected rabbit serum pre-absorbed with mouse cells in the presence of EDTA⁶.

cytotoxic tests on thymocytes (for serological methods see refs. 5 and 6). Other tests to locate θ and *Ly-A* more precisely in linkage groups II and XII are being done.

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- Boyse, E. A., and Old, L. J., *Ann. Rev. Genet.*, **3**, 269 (1969).
- Boyse, E. A., and Old, L. J., The Invitation to Surveillance Conference on Immunological Surveillance, Brook Lodge, Kalamazoo, Michigan (in the press).
- Reif, A. E., and Allen, J. M. V., *J. Exp. Med.*, **120**, 413 (1964).
- Boyse, E. A., Miyazawa, M., Aoki, T., and Old, L. J., *Proc. Roy. Soc.*, B, **170**, 174 (1968).
- Boyse, E. A., Old, L. J., and Chouroulinkov, I., *Methods in Med. Res.*, **10** (edit. by Eisen, H.), 39 (Year Book Med. Publ., Chicago, 1964).
- Boyse, E. A., Hubbard, L., Stockert, E., and Lamm, M. E., *Transplantation*, **10**, 446 (1970).

Role of Different Sponge Cell Types in Species Specific Cell Aggregation

INVESTIGATIONS into species specific sponge cell reaggregation, aimed at illuminating the nature of the adhesive elements at the cell surface and the mechanism responsible, have apparently neglected the fact that a population of dissociated sponge cells consists of several different types which may have dissimilar adhesive properties. Yet the classical studies of reaggregation¹ indicate that some cell types are markedly more active than others in forming aggregates. We have now partially separated the different cell types by density gradient centrifugation and investigated their ability to reaggregate. It seems that the presence of archaeocytes is necessary for aggregation to take place but species specificity in the sorting out process is conferred by mucoïd cells in interaction with the archaeocytes.

We used the monoaxonic sponges *Ophlitaspongia seriata* and *Halichondria panicea* which were collected at Aberystwyth, Cardiganshire, and St Abbs, Berwickshire, and kept in aerated seawater at 4° C and used within 10 days. Monodisperse cell suspensions were obtained by a combination of chemical and mechanical dissociation². Fragments of sponge were washed rapidly with cold ethylenediaminetetraacetic acid disodium salt solution (0.004 M EDTA, 0.55 M NaCl, 0.004 M Tris-HCl, pH 7.2) (ref. 3) and then pressed through 60 XXXX boltsilk into 50 ml. of the same solution at 0° C. The concentration of the resulting cell suspension was determined immediately by means of a haemocytometer and adjusted to 5×10^6 cells per ml. Aliquots (1.0 ml.) were placed on stepwise concentration