associated with the surface of dendritic processes of reticular cells, where it comes in contact with lymphocytes. By contrast, in the medullary sinuses antigen is retained intracellularly in the inclusions of typical phagocytic cells. The distribution of label in lymphoid follicles leads us to suggest that the term 'phagocytic reticulum' which we have previously used⁴ in relation to this area should be replaced by the term 'antigen-retaining reticulum'. It is tempting to speculate that the two patterns of lymph node antigen distribution reflect different facets of the immune processes.

We thank Prof. G. J. V. Nossal and Dr. G. L. Ada for their advice and Mr. J. Pye for performing the iodinations. This work was supported by grants from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service (AI-0-3958), to Prof. Nossel, and from the National Health and Medical Research Council, Australia. Some batches of iodine-125 were a gift of Glaxo Laboratories, England.

JUDITH MITCHELL А. Аввот

Walter and Eliza Hall Institute.

Melbourne, Australia.

- ¹ Ada, G. L., Nossal, G. J. V., Pye, J., and Abbot, A., Nature, 199, 1257, Part II (1963).
- ² Nossal, G. J. V., Ada, G. L., and Austin, C. M., Austral. J. Exp. Biol. and Med. Sci., 42, 311 (1964).
- ³ Ada, G. L., Nossal, G. J. V., and Austin, C. M., Austral. J. Exp. Biol. and Med. Sci., 42, 331 (1964) ⁴ Miller, J. J., and Nossal, G. J. V., J. Exp. Med., 120, 1075 (1964).
- ⁵ Ada, G. L., Nossal, G. J. V., and Pye, J., Austral. J. Exp. Biol. and Med. Sci., 42, 295 (1964).
- ⁶ Palade, G. E., J. Exp. Med., 95, 285 (1952). ⁷ Mitchell, J. (submitted for publication to J. Immunol.).
- ⁸ Mayor, H. D., Hampton, J. C., and Rosario, B., J. Biophys. Biochem. Cytol., 9, 909 (1961).
- ⁹ Salpeter, M. M., and Bachmann, L., J. Cell Biol., 22, 469 (1964).
 ¹⁰ Luft, J. (personal communication).
- ¹¹ Dowell, W. C. T., Fourth Intern. Conf. Electron Microscopy, Berlin, 1958, 1, 375 (1960).

12 Fleming, W., Arch. Mikr. Anat., 24, 550 (1885).

Zinc and Other Metallic lons as Hatching Agents for the Beet Cyst Nematode, Heterodera schachtii Schm.

Some inorganic salts stimulate eggs of the beet cyst nematode, Heterodera schachtii Schm., to hatch, but less effectively than diffusate from sugar-beet roots¹⁻³. The effective ions are potential oxidizing agents. We have extended hatching tests to some metallic ions not previously tried, including Ba²⁺, Al³⁺, Pb²⁺, MoO₄⁻, Mn²⁺, Co²⁺, Zn²⁺ and Cd²⁺, and have re-tested some salts (MgCl₂, KCl, NaCl, HgCl₂ and FeCl₃). Copper, previously tested as the sulphate, was tested as the chloride, and chlorides of the other metals were included in tests whereever possible. All salts were first tested at a concentration of ~ 3 mM in water and those with some activity were tested again over a range of concentrations. The hatch of eggs (Table 1) is expressed as a hatch rating³:

$$\frac{H_s - H_w}{H_d - H_w} \times 100$$

where H_s is the hatch in the substance, H_d is the hatch in beet root diffusate and H_w is the hatch in distilled water, and also as a percentage of the total number of eggs in the cysts.

Zine sulphate, zine nitrate and zine and cadmium chlorides proved potent hatching agents; twelve other salts were moderately or weakly active, and ten were inactive or inhibitory. The zinc salts and cadmium chloride are more effective than the other inorganic hatching agents for H. schachtii³ although neither anion nor cation is a potential oxidizing agent. The activity of these two chlorides (zinc and cadmium) was not caused by hydrochloric acid formed by hydrolysis, because hydrochloric acid itself is only moderately active at its optimum concentration.

Table 1. THE ABILITY OF VARIOUS METALLIC SALTS TO STIMULATE HATCHING OF *H. schachtii* EGGS EXPRESSED AS A HATCH RATING AND ALSO AS A PER-CENTAGE OF THE TOTAL NUMBER OF EGGS IN THE CYSTS

based of total natch after	three weeks from	three bat	ches of 100 cysts
Compound	Concentration (mM)	Hatch rating	Percentage hatch
Zinc chloride	4*	180 *	64
Cadmium chloride	0.6*	177	56
Zinc sulphate	2	127	55
Zinc nitrate	2	113	45
Ammonium molybdate	3*	82	38
Lead acetate	3*	75	23
Aluminium chloride	3*	69	32
Manganese chloride	3*	66	32
Cobalt chloride	0.6*	60	31
Ferrous sulphate	2	59	23
Zinc acetate	2	57	27
Cadmium sulphate	0.6 *	56	34
Barium chloride	3*	54	27
Calcium chloride	3*	53	18
Cadmium nitrate	0.6*	31	22
Ferrous ammonium sulphate	1	26	16
Lead nitrate	2*	22	20
Potassium chloride	3	19	11
Ferric chloride	0.6 *	16	20
Sodium chloride	3	15	11
Cupric chloride	0.6*	4	20
Ammonium nitrate	8	-14t	10
Ammonium sulphate	4	-15	10
Magnesium chloride	3	-30	14
Sodium nitrate	6	- 88	1
Mercuric chloride	0.6*	- 95	1

* Optimum concentration in a dilution series. † Bold type indicates hatch as good as or better than in root diffusate, which is 100 \pm 10. ‡ Negative sign indicates percentage inhibition compared with the hatch in water, which is 0 \pm 10.

Because of its potency in causing H. schachtii eggs to hatch, we tested the ability of zinc chloride to hatch eggs of other Heterodera species: H. avenae, H. carotae, H. cruciferae, H. glycines, H. goettingiana, H. rostochiensis, H. tabacum and H. trifolii. With H. avenae, where the number of eggs which hatch in oat-root diffusate and in water is the same, zinc chloride inhibited hatching slightly. With H. goettingiana, where only very few eggs hatch either in water or in pea-root diffusate in vitro, a few more larvae hatched out in zinc chloride. With all the other species, zinc chloride caused more eggs to hatch than did water, and with most of these species hatching was as great as or greater than that in the root diffusate from their host plants. As with other agents that stimulate hatching, it is not known how Zn²⁺ and Cd²⁺ ions act.

A. J. CLARKE AUDREY M. SHEPHERD

Rothamsted Experimental Station,

Harpenden, Hertfordshire.

¹ Rademacher, R., and Schmidt, O., Arch. PflBau, 10, 237 (1933).

² Wallace, H. R., Ann. App. Biol., **44**, 274 (1956). ³ Clarke, A. J., and Shepherd, A. M., Nematologica, **10**, 431 (1964).

Failure of the Zona Reaction in Five Pig Eggs

In most mammals which have been examined, the first spermatozoon that penetrates into the egg stimulates the egg to undergo a reaction which prevents subsequent spermatozoa from traversing the zona pellucida¹. This phenomenon is called the 'zona reaction'. In the pig, following the zona reaction, spermatozoa can still penetrate into the zona but they normally cannot traverse it². However, eggs aged for several hours before exposure to the first spermatozoon sometimes fail to elicit the zona reaction and several spermatozoa enter the vitellus (polyspermy) to become male pronuclei (polyandry)3.

In the course of examining several hundred recently fertilized pig eggs, we observed five eggs which did not exhibit a zona reaction. The first of these came from a litter of nine eggs recovered about 8 h after ovulation. Eight of these were at normal stages while one egg (egg A) was unusual in that it contained two spermatozoa in the perivitelline space (Fig. 1). After the egg was fixed in acetic-alcohol and stained with orcein, a vesicular nucleus was revealed (Fig. 2). Hancock⁴ reported that out of 1,677 pig eggs examined, three had vesicular nuclei. The second egg that had not undergone a zona reaction came from a litter of five eggs recovered 54 h after insemination.