the (PC+LPC)/SM ratios. These were increased in the recombinates and correspondingly decreased in the upper lipid layer. It is noteworthy that the high affinity for PS shown by both protein fraction when recombined with the single phospholipid was not manifested in the recombinates obtained from total lipid extracts, suggesting that the binding of a individual phospholipid species is influenced by the presence of other lipids.

The phospholipid distribution in the experiments using the PC-SM-cholesterol mixture is given in table 3. PC-SM-cholesterol mixture used in the recombination experiments contained initially 13–15% LPC (see 'Lipid Mixture' table 3), an amount which was not well recovered from either the recombinates or the upper lipid layer. However, since our results (table 2) and those of others[7] show that there is no significiant hydrolysis of PC during the recombination procedure we were able to compare the PC/SM ratios. In 2 experiments with integral proteins and 1 out of 2 with peripheral proteins, we found an increase in PC/SM ratio in the recombinates, paralled by a decrease in the PC/SM ratio in the free lipid upper layer.

Phospholipid distribution in recombinates obtained with a total red cell lipid extract and with a PC-SM-cholesterol mixture showed, for both protein fractions, a preference for binding of PC at the expense of SM. This finding is in agreement with the results obtained by Kramer et al.[7]. The data presented herein confirm the conclusion of Kramer et al.[7] and Wehrli et al.[6] that in membrane recombinates obtained by dialysis from 2-CE solutions, the protein structure is preserved sufficiently to retain lipid binding capacity and specificity.

Delayed-type skin reactions in bursectomized or thymectomized chickens[1]

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Summary. Chickens can easily be induced to develop delayed-type skin reactions to oxazolone when animals are sensitized 7 days before the challenge. The reaction is quantitated by assessing the increase in wattle thickness: maximum reactions occur 24 h after challenge. The reaction is inhibited by neonatal thymectomy or bursectomy; these findings therefore suggest also an important B-derived component in delayed hypersensitivity to oxazolone.

Oxazolone-induced skin reaction in the mouse has proved to be a complex and informative model in elucidating cellular events in delayed-type hypersensitivity. Thymus-derived cells are necessary to induce contact sensitivity and both these and bone-marrow cells, probably monocytes[2], are needed for developing in vivo manifestations of the delayed hypersensitivity. Whereas thymus-derived cells are essential to develop delayed reaction to oxazolone in the mouse[3], there is no definite evidence about a similar function of bone-marrow-derived lymphocytes. Some authors suggested a B-cell participation in this reaction because they observed a proliferation of these cells in the draining lymph-nodes which show germinal center development peaking at 8 days from sensitization[4,5].

The function of this proliferation in the reaction remains unclear, because mice lethally irradiated and reconstituted with B-cells do not develop contact sensitivity to oxazolone[6]. In a preliminary report, we demonstrated by skin test that chickens develop oxazolone hypersensitivity and neonatally irradiated and thymectomized chickens, like thymectomized mice[6], show a depressed skin reaction to oxazolone[7].

The problem of B-cell participation in the delayed-type hypersensitivity can be studied further with advantage in birds, since they possess a separate organ, the bursa of Fabricius, site of production of B-lymphocytes. The results presented seem to show that also the bursa of Fabricius is important in the development of contact sensitivity to oxazolone in chickens.

Materials and methods. Male Hubbard chicks obtained from Nutrix (Palermo) were bursectomized or thymectomized within 5 h after hatching. Some thymectomized chickens, as well as unoperated birds, were irradiated with 550 rads as previously described[8]. Autopsies were performed on all operated birds at the end of the experiment, and chickens showing thymic residues were excluded from the results. Chickens were sensitized with 33 mg/kg of oxazolone (ethoxymethylene-2-phenyl-oxazolone, BDH chemicals Ltd) in 0.5 ml of absolute ethanol applied to the previously shaved anterior region of the neck. Control animals received only absolute ethanol. Chickens were challenged 7 days later with 25 mg of oxazolone in olive oil applied to the 2 wattles. The reaction was tested with a screw gauge micrometer by measuring the increase of the wattle thickness at 6, 24 and 48 h after challenge. Wattle thickness was expressed as percent average of increase. Results were analyzed by Student's t-test.

Results and discussion. Our initial experiment reconfirms the previous finding[7] that chickens can be easily induced to develop contact sensitivity by painting the wattles with the sensitizing agent, and quantitated by assessing the increase in wattles thickness; maximum reactions occur 24 h after challenge, smaller increases in wattles thickness are also detected 6 and 48 h after challenge. Reactions are characterized by wattle swelling accompanied by slight ischemia and occasionally at 48 h by detachment of skin and/or necrosis. By contrast, oxa-

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zolone concentrations used are found to have virtually no effect (less than 10% of increase) in non-sensitized animals on wattles thickness. The results suggest that the main reaction observed in sensitized chickens are of the delayed type: this view is supported by the histological examination of the challenge site (our unpublished observation[9,10]), the peaking at 24 h after challenge and the demonstration that thymus has a function in the induction of this reaction (figures 1, 2).

Thymectomy alone or with irradiation indeed significantly suppresses the response to oxazolone; irradiation itself does not produce a significant inhibition (figure 2). It is interesting to note that irradiation alone is slightly inhibitory (column C), while irradiation of thymectomized chickens (column B) is not cumulative to the effect of thymectomy (column A): thymectomy alone seems to be more suppressive without irradiation. The results obtained in thymectomized animals at various ages (9 and 12 weeks) demonstrate that there is a gradual recovery of T-cell functions, so thymectomized animals at 9 weeks of age (figure 2) are relatively incapable of producing a delayed-type reaction, but at 12 weeks of age they had almost completely recovered (figure 1).

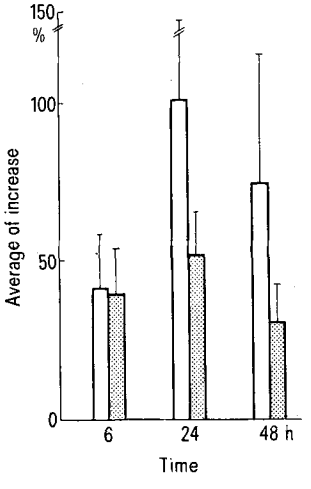

Fig. 1. Effect of thymectomy and irradiation (diagonally hatched bars) on increase in wattle thickness after challenge with 25 mg of oxazolone; birds were sensitized at 12 weeks of age. Increase in wattle thickness was recorded 6, 24 and 48 h after challenge and was expressed as percent average of increase. The bars represent mean ± SE in groups of 4–6 chickens.

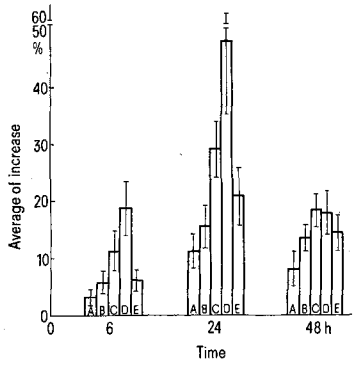

Fig. 2. Effect of thymectomy alone (A) or with irradiation (B), irradiation alone (C), none (D) or bursectomy (E) on increase in wattle thickness after challenge with 25 mg of oxazolone 7 days after sensitization (at 9 weeks of age). Increase in wattle thickness was recorded 6, 24 and 48 h after challenge and was expressed as percent average of increase. The bars represent mean ± SE in groups of 5–10 chickens. Statistical significance was observed versus control at 24 h in groups A ($p < 0.02$), B ($p < 0.025$) and E ($p < 0.05$).

Experiments depicted in figure 2 also show that neonatal bursectomy influences the skin test to oxazolone: chickens bursectomized just after hatching show a significantly reduced response to oxazolone (column E). Our results suggest that contact sensitivity to oxazolone in chickens is modified by neonatal bursectomy, thus not confirming previous reports indicating that delayed hypersensitivity was not affected in hormonally bursectomized chickens[11,12]. The reasons for this discrepancy of our results with earlier reports might well depend on the antigen and the strain of chickens used: in chickens a genetic determination of hypersensitivity reactions has been demonstrated[13].

Recently Weidanz et al.[10] reported that allergic contact dermatitis to oxazolone was not inhibited by chemical bursectomy: on the contrary, the reaction seemed to be augmented in bursectomized animals. In contrast to those studies, we used a shorter timing between sensitization and challenge, higher doses of oxazolone and surgical instead of chemical bursectomy. In our system we do not have a B-cell depleted animal[14], although surgical bursectomy certainly interfers with B-cell traffic to thymus[15] and bone marrow[16] and decreases suppressive cells of bursal origin in the thymus[17].

In summary, the results presented clearly show that delayed-type skin reaction to oxazolone is inhibited in chickens by neonatal thymectomy or bursectomy. Our findings suggest an important B-derived component in the reaction to oxazolone, although the experiments do not clarify whether bursa cells are involved directly in the response to oxazolone.

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