in the reaction, thus indicating that the blockade of rosette formation was due to a specific anti-Ig component of the antiserum. The inhibition was less effective in the case of spleen and bone marrow.

Discussion. In the chicken, the appearance of RFC is considered a bursa-dependent phenomenon[3]. However, the most interesting finding of this study is the striking decrease in the number of lymphocytic RFC in the bursa, spleen and bone marrow of thymectomized birds thus suggesting the existence of a thymus-dependent RFC. The T rosettes[4], and 2 populations of thymus-derived RFC, 1 of them being influenced by thymectomy[5], have been described in mice. Rosette formation by T cells was also inhibited by anti-Ig reagent[6]. The involvement of the chicken thymus in the formation of rosettes can be explained in at least 2 ways. The first possibility is than T cells interact with bursa-dependent cells (Bu cells) it rosette reactions so that T cells exert a helper function whereas Bu cells establish a more substantial contact with erythrocytes[7]. The second assumption is that T cells are themselves capable of forming rosettes, and this is probably the case with lymphocytic rosettes in bursaless birds. Both explanations, of course, concern RFC in non-immunized chickens, and one may expect a somewhat different situation in birds immunized with GPRBC.

Although it has been stated that natural RFC constitute progenitors of antibody-producing cells[8], the immune nature of those rosettes is still unclear. As has been proposed for natural haemagglutinins and haemolysins[9], the natural RFC may also arise from previous contacts of the animal with cross-reacting antigens, or their appearance is under genetic control.

Ig receptors have been demonstrated on the surface of chicken Bu cells[3,10]. However, the existence of Ig receptors on mammalian and avian T cells is the subject of divergent views, since both the presence[5,11] and the absence[12] of Ig receptors have been reported. Although our results favour the former view, it still remains to be seen whether natural lymphocytic rosettes in the chicken are an expression of Ig-like receptors on lymphocytes[13], or a special class of 'physiological' rosettes similar to those which human T cells form with sheep erythrocytes[14].

The possibility that T rosettes may be formed 'pasively' via a cytophilic antibody elaborated by Bu cells[15] seems unlikely since there was a clear difference between spleen, marrow and thymus lymphocytes of bursectomized birds with respect to their ability to produce rosettes. Finally, the formation of rosettes by granulocytes and erythroblasts remains an event which can be treated as a physico-chemical, a physiological, a developmental or an immunological phenomenon.

Résumé. On démontre la présence des lymphocytes capables de faire des rosettes spontanées ou naturelles avec les globules rouges de cobaye, dans le thymus, la bourse de Fabricius, la rate et dans la moelle osseuse des poulets non-immunisés, âgés de 8 semaines. Les effets de la boursectomie et de la thymectomie néonatale chez le poulet, suggèrent l'existence de 2 populations de lymphocytes capables de faire des rosettes, dont l'une thymo-dépendente et l'autre dépendant de la bourse.

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The Location of Soluble Antigen in the Spleen of *Xenopus laevis*

The pattern of antigen localization in the spleen has been traced in rats[1], *Bufo marinus*[2] and mice[3] using flagellar antigens or aggregated human γ-globulin (HGG). It is known from previous studies by Horton and Manning[4] using a fluorescence technique to detect antigen, that *Xenopus laevis* – an animal lacking in lymph nodes – can trap antigen in its spleen. They found that 3 weeks after injecting HGG in adjuvant, the fluorescent picture was diminished or absent in thymectomized toadlets. In this report, the location of antigen in spleens from intact and thymectomized *Xenopus* injected with HGG in saline was investigated at various time intervals after injection. The antigen was traced using a fluorescein labelled antiserum to HGG.

Materials and methods. The experimental animals (weighing approximately 8 g and maintained at 20 °C) consisted of 56 intact, 10 thymectomized and 7 sham-thymectomized toadlets. For details of the thymectomy operation at larval stage 48 of Nieuwkoop and Faber[5] see Horton and Manning[4]. A standard solution of 5 mg of HGG (Kabi, Stockholm) in 1 ml of 0.85% saline was prepared and injected in various volumes via the dorsal lymph sac so that toads received 0.1, 0.15, 0.6 or 1.5 mg HGG in 1 injection. 28 control animals received saline only. Toads were sacrificed at 1, 6, 12 and 24 h, then daily for 1 week then every 2 weeks for 10 weeks after injection. The spleen was removed, quick-frozen in liquid nitrogen and sectioned at 10 µm on a cryostat. The immunofluorescent technique for the detection of antigen[4] was applied to the spleen sections. Serum, from toads killed 8 or 10 weeks after receiving 0.6 or 1.5 mg HGG was tested for

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anti-HGG antibodies by a passive haemagglutination technique previously used by TURNER and MANNING[6].

Results and discussion. The description of the location of fluorescence in the spleen (illustrated in Figures 1–3) is as follows: 30 min after injection of 0.15 mg HGG, antigen was located in the red pulp of the spleen surrounding the white pulp area (Figure 1); 30 min later, the beginnings of a new area of fluorescence inside the white pulp boundary was seen. 6 h after injection, fluorescence in both areas could be seen in equal intensity (Figure 2) and this situation persisted for several hours until at 2 days after injection the fluorescence in the white pulp looked slightly brighter than that in the red pulp. At 3 days post injection, the red pulp fluorescence was beginning to disappear and had done so completely by 4 days leaving only the fluorescence in the white pulp area (Figure 3). This picture had disappeared by 10 weeks. At a lower dose (0.1 mg HGG), the same sequence of events occurred but at a different rate. Red and white pulp fluorescence was not visible simultaneously until 6 h post injection and the red pulp fluorescence was disappearing by 12 h and absent 24 h after injection. 1 day later, the number of fluorescent areas was diminished, though the intensity of the areas that did fluoresce was as high as at earlier times. Spleens tested for fluorescence were negative 4 days after receiving this dose.

Antibodies to HGG were not detected in the serum until 10 weeks after injection when the fluorescence had either disappeared or was very faint and scattered in the white pulp. At this time anti-HGG titres were low and not comparable with titres obtained at 2 weeks after injection with HGG in complete Freund's adjuvant[7] when white pulp fluorescence was present or at 8 weeks when fluorescence was disappearing[4]. It is not known whether there is any direct correlation between antigen location in the spleen and serum antibody production in *Xenopus*. However, some circumstantial evidence does exist: thymectomized *Xenopus* show a diminished fluo-

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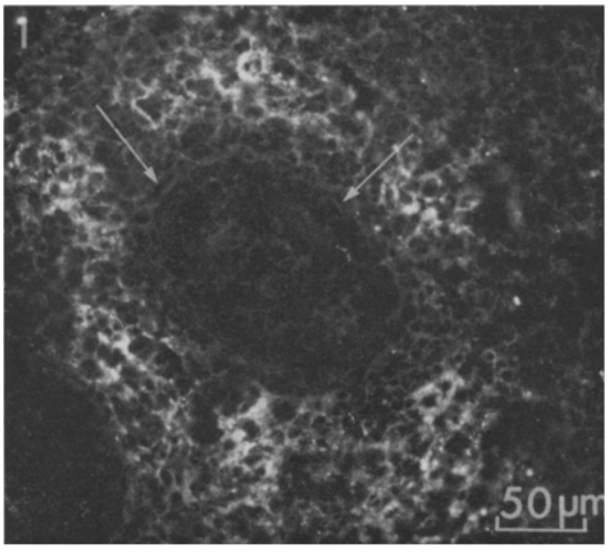

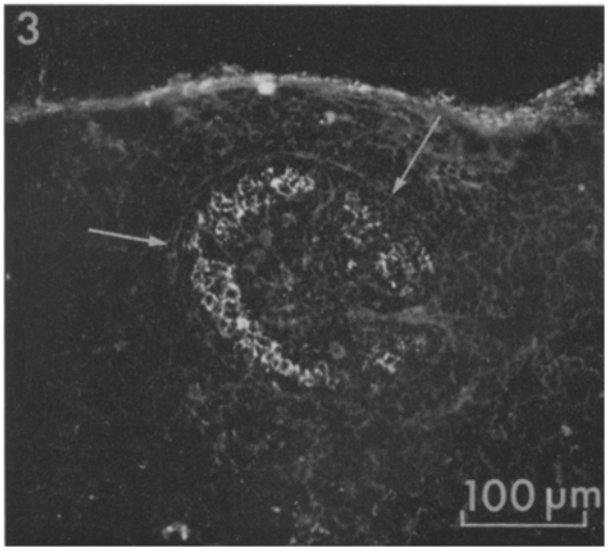

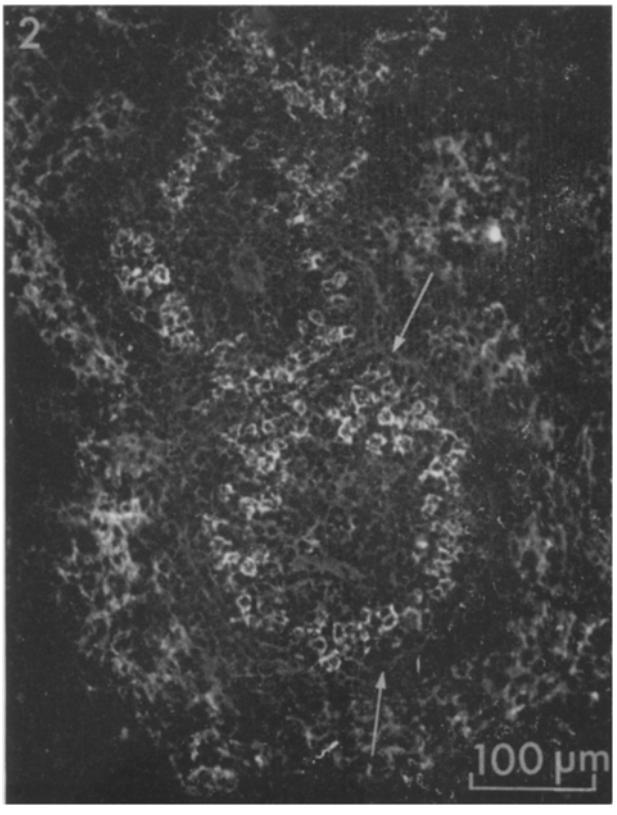

Figs. 1–3. Immunofluorescence preparations of spleen sections from toads given HGG and killed at various time intervals after injection. Fluorescence indicates the presence of antigen. Arrows point to the boundary of the white pulp island. 1. (30 min after injection) shows fluorescence appearing only in the red pulp encircling the boundary of the white pulp island. 2. (6 h post injection) demonstrates fluorescence in both the red pulp and white pulp. 3. shows the later location of fluorescence when it appears inside the boundary of the splenic white pulp only.

rescent picture[4] and they are unable to mount an antibody response to HGG in adjuvant[6]. In the present experiment, thymectomized toadlets given 0.15 mg HGG and killed at 6, 12, 24 h or 5 weeks after injection, showed the same fluorescent picture as intact animals at 6, 12 and 24 h. However, 5 weeks after injection, fluorescence was severely diminished in thymectomized animals compared to intact animals. Using HGG in adjuvant, HORTON and MANNING[4] also found specific fluorescence diminished or absent in thymectomized toadlets 3 weeks after receiving the antigen.

The pattern of antigen localization in *Xenopus* was very similar to that observed by NOSSAL et al.[1] using radioactively labelled polymerized flagellin in rats. BROWN et al.[3], tracing aggregated HGG in rat spleens by immunofluorescence also emerged with a picture similar to the one described here in *Xenopus*. They postulate that spleen based lymphocytes carry the antigen into the white pulp follicle. It is not known whether the presence of fluorescence in the white pulp of the *Xenopus* spleen shortly after being seen in the red pulp, signifies a real or apparent movement of antigen. TURNER and MANNING[8] have shown that there are places where the boundary layer surrounding the white pulp region is indistinct, where presumably cells could pass from one region to the other.

The initial appearance of antigen around the periphery of the white pulp island is reasonable when one considers that it is in this area that blood sinuses are found[9]. Carbon, a non-antigenic material, first becomes visible in the *Xenopus* spleen in the same position in the red pulp as HGG[10]. Later on carbon appears in the white pulp but is mainly grouped around the central arteriole. Also, in rats[1] and *Bufo marinus*[2], carbon is first seen in the red pulp in the spleen though its later localization differed from that shown with flagellar antigens. This information, taken with the knowledge that the red pulp fluorescence appears within 30 min of injection, indicates that the area surrounding the red pulp island is the place where material injected via the dorsal lymph sac first enters the spleen – presumably via the blood stream. Thymectomy appeared to have no effect on the early stages of antigen trapping. This, together with TURNER's carbon studies[10], suggests that the initial localization of foreign material in the spleen does not involve the immune mechanisms which become evident at later stages.

Résumé. Une étude de la localisation de γ-globulines humaines (en solution saline) dans la rate de *Xenopus laevis* a été effectuée en utilisant la fluorescence pour la détection d'antigènes. La thymectomie n'a eu aucun effet sur la première apparition d'antigènes dans la pulpe rouge entourant la zone de pulpe blanche.

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Promotion of Antibody Formation by Prostaglandin

Prostaglandins are known to be involved in miscellaneous membrane-associated events and the effects on the immunocompetent cells have been reported[1–3]. However, there have been no reports on the effect on antibody formation. As reported in a previous paper[4], we found that a low dose of an antibiotic named diketocoriolin B promotes antibody formation. This antibiotic has some structural resemblance with prostaglandins, and therefore we undertook the study on the effect of prostaglandin on antibody formation.

As presented in this paper, it was found that a low dose of prostaglandins E_1, E_2, $F_1\alpha$ and $F_2\alpha$ promotes primary and secondary antibody formation to sheep red blood cells in mice.

Female *dd/Y* mice (5–6 weeks old, weighing 20–22 g) were immunized to sheep red blood cells (SRBC), and the

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Table I. Effect of prostaglandins (PG) on primary antibody formation

	Average No. of PFC $\times 10^3$/spleen 4 days after immunization	
	Exp. I	Exp. II
SRBC 10^8 i.v.[a]	59.3 ± 7.0	62.3 ± 5.9
SRBC 10^8 + PG E_1 1 µg[b] i.p.	91.0 ± 16.3	
SRBC 10^8 + PG E_1 0.1 µg i.p.	171.3 ± 31.5	139.0 ± 3.1
SRBC 10^8 + PG E_2 1 µg i.p.	186.0 ± 28.0	
SRBC 10^8 + PG E_2 0.1 µg i.p.	214.0 ± 25.3	210.0 ± 5.5
SRBC 10^8 + PG $F_{1\alpha}$ 1 µg i.p.	115.2 ± 28.8	
SRBC 10^8 + PG $F_{1\alpha}$ 0.1 µg i.p.	88.2 ± 7.4	151.5 ± 2.0
SRBC 10^8 + PG $F_{2\alpha}$ 1 µg i.p.	147.0 ± 23.1	
SRBC 10^8 + PG $F_{2\alpha}$ 0.1 µg i.p.	101.7 ± 7.0	234.7 ± 1.3
None	0.26 ± 0.08	0.21 ± 0.04

[a] SRBC in 0.1 ml/mouse. [b] Each dose of PG in 0.2 ml/mouse.