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.

Table II. Effect of prostaglandins (PG) on secondary antibody formation

Primary immunization	Average No. of PFC/spleen 28 days after the primary immunization	Average No. of PFC/spleen 4 days after the secondary immunization
SRBC 10^5 [a], i.v.	6.7 ± 1.2	3675 ± 230 [c]
SRBC 10^5 [a] + PG [b] E_1, 0.1 µg i.p.	5.0 ± 0.8	8717 ± 203
SRBC 10^5 [a] + PG E_2, 0.1 µg i.p.	6.7 ± 1.8	15783 ± 1603
SRBC 10^5 [a] + PG $F_{1\alpha}$, 0.1 µg i.p.	20.0 ± 4.0	20117 ± 3083
SRBC 10^5 [a] + PG $F_{2\alpha}$, 0.1 µg i.p.	12.5 ± 1.4	20317 ± 3492
None [d]	20.0 ± 2.8	68.3 ± 7.8
None	—	18.8 ± 2.1

[a] SRBC in 0.1 ml/mouse. [b] PG in 0.2 ml/mouse. [c] The number of PFC without the second immunization was 7.0 ± 0.8. [d] Secondary immunization alone.

number of plaque-forming cells (PFC) was counted by a hemolytic plaque method[5]. Five mice were used for each group. Prostaglandins E_1, E_2, $F_1\alpha$ and $F_2\alpha$ were supplied by Ono Pharmaceutical Co., Osaka, Japan. 500 µg of each prostaglandin was dissolved in 0.05 ml of dimethylsulfoxide (DMSO) and 0.95 ml of saline was added. This solution was diluted with saline to the desired concentration and 0.2 ml was injected to a mouse i.p.

In one experiment, 10^8 SRBC were i.v. injected to mice and at the same time 1.0 or 0.1 µg of each prostaglandin was injected i.p. The number of PFC was counted 4 days thereafter. As shown in Table I, all prostaglandins increased the number of PFC. Comparing with antigen alone, the antibody formation was stimulated about 2–4 fold. The vehicle containing 0.1–0.01 µl of DMSO did not affect antibody formation.

The effect of prostaglandins on secondary antibody formation was shown by another experiment. 8 mice in each group were primed by i.v. injected of 10^5 SRBC with or without simultaneous i.p. injection of 0.1 µg of each prostaglandin, and the number of PFC of 3 primed mice in each group was checked 28 days after the primary immunization. Thus, it was ascertained that the number of PFC was the same as that of the non-treated control, that is, the effect of primary immunization disappeared. Then, the second immunization was made by i.v. injection of 10^5 SRBC, and the number of PFC was determined 4 days thereafter. As shown in Table II, in comparison with the number of PFC of mice primed with antigen alone, mice primed with antigen and prostaglandin showed 2.4–5.5 times larger number of PFC.

The stimulatory effect of prostaglandins on antibody formation was also observed in spleen cell culture in vitro. The procedure described by MISHELL and DUTTON[6] was closely followed. At the initiation of culture of spleen cells from CDF_1 mice SRBC was added with or without 1.0 pg of each prostaglandin. The number of PFC was determined 4 days thereafter. The result, which indicates the significant increase of the number of PFC in the culture with each prostaglandin, is shown in Table III. Thus, it is clear that each prostaglandin affects the immunocompetent cells directly.

Table III. Effect of prostaglandins on antibody formation in vitro

Cultures supplemented with	PFC [c]/10^6 recovered cells 4 days after start of cultures
SRBC [a]	329.7
SRBC + PG [b] E_1	662.3
SRBC + PG [b] $F_{1\alpha}$	608.7
SRBC + PG E_2	560.8
SRBC + PG $F_{2\alpha}$	456.4

[a] Sheep red blood cells: 1×10^7 cells were added to 15×10^6 spleen cells in culture. [b] Prostaglandin: 1 pg/culture. [c] The number (13.7) of PFC of SRBC-free control was subtracted.

Membrane-associated functions in the immunocompetent cells play an important role in antibody formation, and various agents which affect membrane-associated events are known to influence antibody formation[7–9]. As reported in a previous paper[4], diketocoriolin B, which inhibits Na-K-ATPase and affects cell membrane[10], stimulates antibody formation, when a very low dose, such as 0.1 µg, is injected i.p. to mice, or 10^{-10} g is added to the culture of antibody-forming system. As will be published, diketocoriolin B was suggested to have an affinity to thymus-derived cells. Moreover, there is a report which described the binding of prostaglandin E_1 to thymocytes[11]. The data described in this paper indicate that a very low dose of prostaglandins enhances antibody formation in vivo and in vitro, and suggest the involvement of prostaglandins in defence mechanism.

Zusammenfassung. Nachweis, dass auch bei geringer Dosis die Prostaglandine E_1, E_2, $F_1\alpha$ und $F_2\alpha$ sowohl die primäre als auch die sekundäre Antikörperbildung in Mäusen erhöhten. Die gleiche Erhöhung konnte auch in der Milzzellenkultur beobachtet werden.

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