by heterologous anti-globulin serum appeared only after the 7th day[18]. Thus, the first PFCs appear to represent a homogenous population of IgM producing cells and circulating antibody probably has no direct regulatory role in their stepwise appearance. It seemed likely that the initial appearance of PFCs was due to a non-random process of recruitment of PFCs from a pool of as yet uncharacterized precursors.

To test the importance of cellular proliferation in this response cytosine-β-D-arabinofuranoside HCL (CA) was used as a specific inhibitor of DNA synthesis[19,20]. For this purpose CA was injected repeatedly during the 1st, 2nd or 3rd day after immunization into parallel groups of mice. The number of PFCs per spleen was determined at 48 and 96 h (Table). When CA was injected between 6 and 24 h after immunization there was only a moderate depression in the number of PFC detected at 48 h. However, when CA was injected between 24 and 36 h the number of PFCs detected at 48 h was almost completely suppressed. Injections of CA between 48 and 72 h also completely suppressed PFC formation at 96 h (Table).

Thus, it seems reasonable to conclude that an antigen induced DNA synthesis and proliferation of cells occurs mainly during the second day of the latent period and that this proliferation is essential for both specific differentiation of antibody forming cells and their accumulation. On the other hand, the nature of this proliferation is by no means clear. It seems highly unlikely that the first PFCs arise by a symmetric division of some hypothetical precursor cells during the latent period, since even 1 such precursor cell, with a generation time of 5–7 h[21–24], would give a higher number of PFC progeny than that found experimentally. Furthermore, the 'staircase' type of cell accumulation to sheep erythrocytes described recently by others[23] also would not reflect a simple clonal 'growth' of PFCs. Therefore, the results of this study support the concept that antibody forming cell differentiation is the result of more complicated cellular processes, including cell interaction[24–26] and recruitment from a compartment of proliferating precursors[1–3,21–23].

To overcome the difficulties due to natural immune background, STERZL et al.[27] and KIM and WATSON[28] have resorted to the use of germ-free precolostral piglets to study the earliest phase of differentiation of hemolytic PFCs to sheep red blood cells. Similar to the results described in this study with conventionally bred mice lacking a pre-existing background to cholera bacilli, they also found a definite latent period of approximately 2 days before first appearance of hemolytic PFCs. Thereafter there was a rapid increase in the number of PFCs, generally greater than that which could be accounted for by simple cellular proliferation. The consistency of the latent period of immune response to 2 unrelated antigens in experimental systems without a natural immune background suggests that a specified time period may be necessary for certain cellular events initiated by antigen stimulation before the first antibody can be synthesized or released[29].

Zusammenfassung. Nachweis, dass «Plaque»-bildende Antikörperzellen gegen somatische Antigene von *Vibrio cholerae* sich in der Milz immunisierter Mäuse schnell und «treppenartig» nach übereinstimmender Latenzzeit von 42 h entwickeln, ohne dass dabei ein «background» an Antikörper bildenden Zellen zu erfassen war.

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Bile Salts and Platelet Aggregation

Relatively large amounts of taurine are known to be present in human platelets[1]. Since taurine is present in bile conjugated with bile salts, it seemed to us not unreasonable to consider the heretofore unsuspected possibility that some platelet taurine might be conjugated with bile salts which might play a role in platelet aggregation. It it not known, however, whether bile salts influence the aggregation of platelets. We report here on the effect of bile salts on platelet aggregation and plug formation in human citrated platelet-rich plasma (PRP).

Platelet aggregation and plug formation was studied in the rotating loop system as previously described[2] except that the blood was collected into 2.69% trisodium citrate. All bile salts were purchased from Calbiochem, Los Angeles, California, and were labelled Grade A. In 15 experiments (9 different plasmas) it was observed that rapid platelet aggregation and plug formation always occurred in response to sodium deoxycholate (DOC) at a final concentration of 1.7 mM and in several cases to as little as 0.43 mM. Several taurine conjugates of bile salts were also studied and found to cause platelet aggregation. All were less active than DOC. Their activity in descending order was: sodium taurodeoxycholate > sodium taurochenodeoxycholate > sodium taurocholate (Table). Free taurine had little or no influence on platelet aggregation (Table).

During the course of these studies it was noted that at a fixed concentration of DOC the aggregation parameters improved as the interval between obtaining the blood sample and performing the test was increased. Qualitatively similar results were obtained with sodium taurodeoxycholate and sodium taurochenodeoxycholate.

This increase in the potential for platelet aggregation and plug formation in normal, human PRP in response to bile salts is similar to that previously reported for low concentrations of adenosine diphosphate[3] and calcium ions[2].

Since PRP is always contaminated with variable numbers of red blood cells, erythrocyte counts were done on all PRPs and were found to be less than 1% of the platelet count. Thus a PRP containing 400,000 platelets/μl contained less than 4000 erythrocytes. If the average ADP content is assumed to be $4 \times 10^{-6} M/10^{11}$ red cells[4] and if all the contained ADP were released into the test system then the final concentration of ADP (contributed by erythrocytes) would be of the order of $10^{-8} M$. This amount of ADP does not cause rapid platelet aggregation and plug formation (Table). However, it might be argued that 'low density' red cells remaining in PRP might represent a select portion of the total red cell population with very high ADP content. For this reason it is not possible at this time to fully exclude the possibility that components released from red cells may contribute to platelet aggregation initiated by bile salts.

Extracts of freeze-dried human platelets have been examined by gas-liquid chromatography in the laboratory of Dr. D. KOWLESSAR. The preliminary findings indicated peaks with retention times similar to those obtained with standards of deoxycholic, chenodeoxycholic and cholic acid and in amounts greater than could be accounted for by contamination with plasma. Further studies will be necessary before final identification of these peaks can be achieved.

The data in this report indicate that exogenous bile salts can cause platelet aggregation and that bile salts may be present within or on platelets. An intensive search for bile salts or bile acids in platelets appears desirable.

Effect of bile salts[a] on platelet aggregation[b]

Substance tested		Seconds to produce First particles	Snow storm	Large aggregates	'Platelet plug'
Sodium deoxycholate		12	19	23	28
Sodium taurodeoxycholate		20	25	30	[c]
Sodium taurochenodeoxycholate		25	30	35	[c]
Sodium taurocholate		25	35	50	[c]
Controls	Taurine ($8 \times 10^{-3} M$)	35	[c]	[c]	[c]
	ADP ($1 \times 10^{-8} M$)[d]	50	[c]	[c]	[c]
	Buffered saline	[c]	[c]	[c]	[c]

[a] Each bile salt was tested at a final concentration of $7.5 \times 10^{-4} M$. [b] Details of the test system are given in reference[2]. In brief, 1 ml of PRP was added to a plastic loop. This was followed rapidly by 0.1 ml of buffered saline solution. The loop was closed and the contents mixed for 1 min by rotating at 12 rpm. Bile salt or control solution (0.1 ml) was added, the loop was closed and rotated again. A stop watch was started to time the occurrence of the aggregation parameters. The PRP was kept in siliconized glass tubes at 20 °C and all tests were run at this temperature. [c] The event did not occur. [d] Included as a control to show that ADP which could possibly be released from small numbers of erythrocytes cannot account for the potent effect of bile salts on platelet aggregation.

Zusammenfassung. Nachweis, dass der Zusatz von Gallensäure die Aggregation menschlicher Plättchen im Plasma in vitro herbeiführen kann und dass die Säure selbst in den Plättchen vorhanden sein dürfte.

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Survival of Embryonic Limb Bud Transplants in Snapping Turtles[1]

Embryologists have shown that allogeneic and xenogeneic tissue can be transplanted successfully between ectothermic vertebrate embryos[2]. The ultimate fate of such grafts received little attention until the discoveries of histocompatibility antigens and immunological tolerance. During the past decade there has been some clarification of the relationship between immunological tolerance and the degree of genetic disparity between amphibian donor-host combinations[3–6]. On the other hand, others reported wide ranges of reactions, from tolerance to rapid rejection[7], rejection of xenografts in one direction, tolerance in the other[8,9] and rejection or tolerance depending on graft dosage[10,11].

A recent article from our laboratory reported the fate of embryonic transplants in two xenogeneic turtle combinations. The rejection of *Amyda ferox* parts (pigment and carapace rudiments) by *Chelydra serpentina*, occurred a few months after hatching. However, partial or complete acceptance for up to 4 years of *Chysemys picta* parts by *Chelydra serpentina* was found in all cases[12]. The studies in the present report involve limb bud transplants, whose viability or destruction can be assessed more accurately than that of less conspicuous embryonic rudiments. These studies were designed to investigate the fate of allogeneic and xenogeneic embryonic transplants in the snapping turtle, *Chelydra serpentina*.

Materials and methods. Stocks of snapping turtles, *Chelydra serpentina*, and painted turtles, *Chrysemys picta*, with fertile eggs were obtained in early June from the Lemberger Company in Oshkosh, Wisconsin, USA, and from the Oak Orchard Conservation Area in central New York, USA. They were fed whitefish once a week and were maintained in a large tank containing 3–4 inches of water at about 20 °C until the procurement of their eggs.

The methods of procurement of turtle eggs, the general operative procedures, and the post-operative care used in this work were those described in a previous report[13].