50–90 days from imaginal ecdysis. Thereafter sporadic active females appeared. After the transfer of young diapausing females to long-day conditions, the median preoviposition lasted 21 days in selected strain, while that of nonselected strain lasted 22–24 days. No substantial difference in diapause intensity between different geographic populations was observed[5]. The slight decrease of diapause intensity in the selected strain is not very important since the median preoviposition (from imaginal ecdysis) of nondiapause individuals of both strains in long day conditions is only about 8–10 days (figure 2).

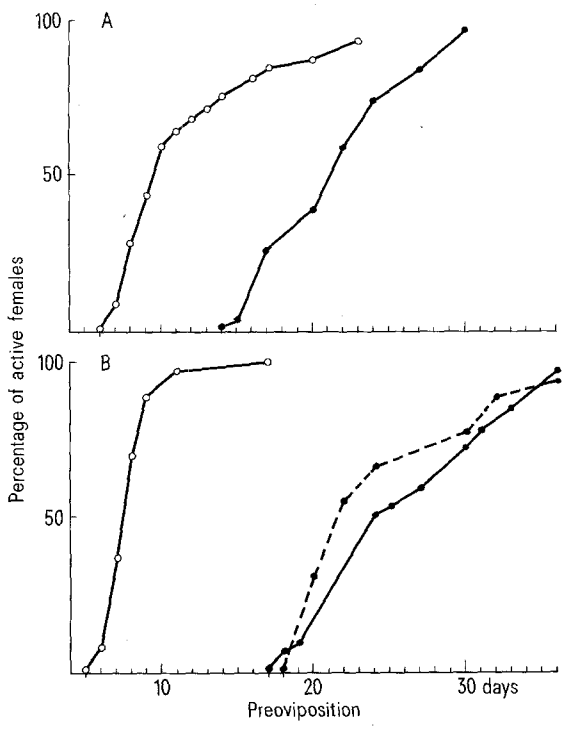

Fig. 2. Duration of preoviposition (from transfer to long day in diapausing and from imaginal ecdysis in nondiapausing females resp.). *A* Selected macropterous strain (28th generation; ●——● diapausing females (N = 31), ○——○ nondiapausing females (N = 32). *B* Nonselected material (2nd generation); ●——● diapausing females from Bohemia (N = 31), ●----● diapausing females from Central Asia, Alma-Ata (N = 18), ○——○ nondiapausing females from Bohemia (N = 36).

The possibility of changing the photoperiodic reaction by selection was demonstrated, with respect to diapause, several times[6–10]. Also the systems responsible for polymorphism in flight organs and/or flight performance are capable of selection[11,12]. In our experiments, probably for the first time, the originally homogeneous photoperiodic reaction of 2 characters has been separated by selection. Theoretically, selection may operate at several levels: the level of sensory receptors, the level of CNS elaboration of environmental cues[13], the level of humoral mediators[14,15] or the level of sensitivity of target organs. In the macropterous strain of *P. apterus,* evidently the capacity to perceive the photoperiodic signal and to elaborate it into the endocrine impulses for ovariole ripening was not disturbed. Thus, either the hormonal mechanism controlling wing polymorphism and that controlling diapause must be different, or the selection must operate at some lower level, presumably that of the sensitivity of target organs (i.e. groups of cells destined to develop into wing membranes).

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Binding of cytophilic rabbit IgG to homologous hepatocytes

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Summary. Rabbit liver cells were able to bind cytophilic monomeric and polymeric homologous IgG via their Fc receptor binding sites (FcR). On the other hand, non-cytophilic rabbit IgG did not bind to hepatocytes, even after its aggregation. The present findings suggest that FcR on rabbit liver cells are specific for cytophilic monomeric IgG but do not significantly bind non-cytophilic, polymeric IgG.

It has recently been reported that human and rabbit hepatocytes are able to attach allogenic and xenogenic IgG via their FcR. The results have shown that heat-aggregated IgG or antigen-IgG antibody complexes are attached to more than 90% of the hepatocytes, while monomeric IgG (7 S) is bound to less than 10% of the treated cells[2]. The attachment was revealed by immunofluorescence using a sandwich technique with non-labelled IgG and fluorescent anti-IgG antibody. However the fluorescent staining data were contradicted by the lack of rosette formation between hepatocytes and sheep red blood cells (SRBC) coated with IgG anti-SRBC (EA)[2]. These controversial results might be explained by the assumption that the hepatocytes have only FcR for monomeric IgG (the so-called cytophilic IgG[3]). Thus the binding of heat aggregated IgG or soluble antigen-antibody complexes is exclusively accomplished by the

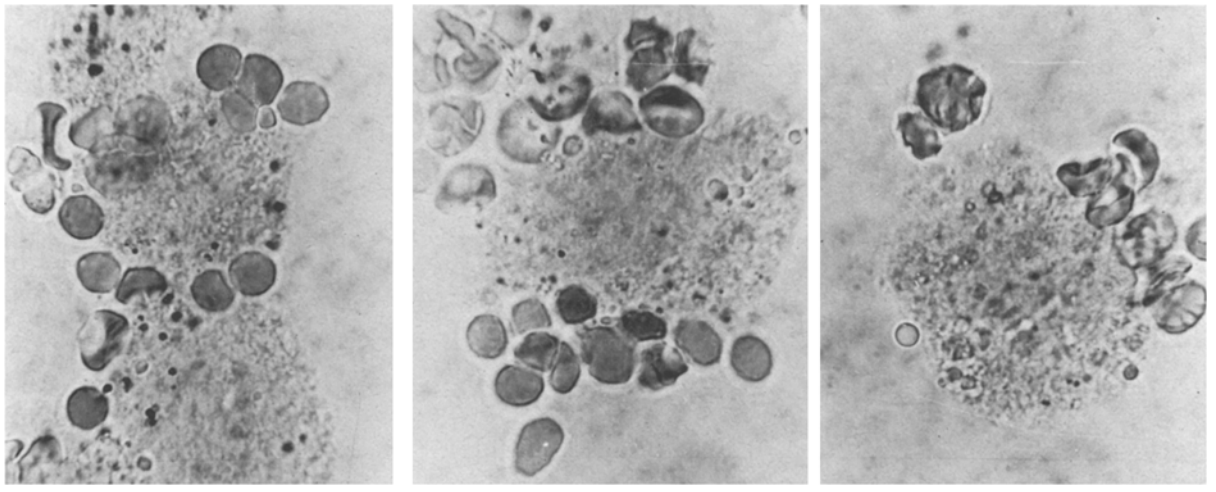

Fig. 1. Photomicrograph of ES rosettes formed by rabbit hepatocytes treated with rabbit IgG.

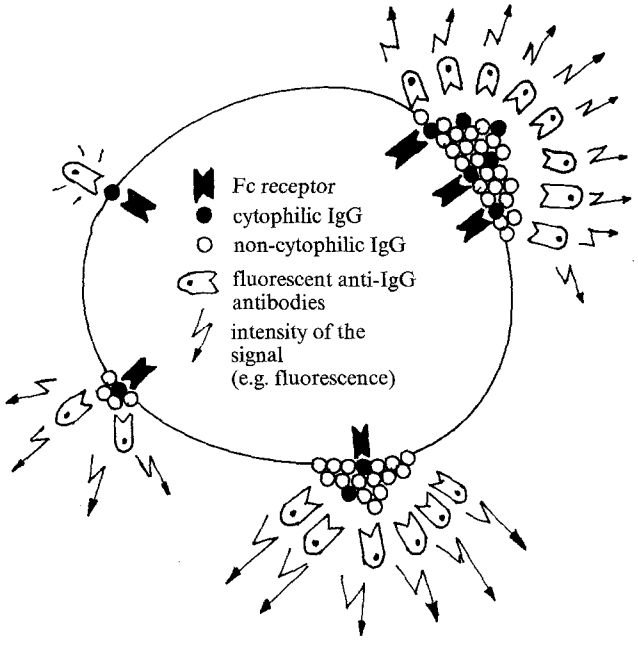

Fig. 2. Tentative model for the binding of IgG, monomer and aggregate to rabbit hepatocytes and the ability to detect their presence on the cell surface by immunofluorescence staining. The low amount of monomeric cytophilic IgG bound to the cell surface cannot be properly detected by the fluorescent anti-IgG antibody. Only the presence of IgG aggregates on the cell surface can be visualized by the fluorescent reagent.

fixation to FcR of the cytophilic IgG molecules present in these aggregates, irrespective of their molecular size or conformation. The present study provides evidence supporting this point of view.

Material and methods. The hepatocytes were prepared from rabbit liver according to Hopf et al.[4]. The cells, incubated before any treatment for 2 h at 37 °C (2×10^6 cells ml^{-1} in TC-199 medium with 2% bovine serum albumin[5]), had a viability over 90%. Macrophages were harvested from washing of rabbit lungs[6] and had a purity of 90–95%. Rabbit IgG (rIgG) was prepared from normal sera by DEAE-cellulose chromatography and gel filtration on Sephadex G-150. Fab and Fc fragments were prepared by papain digestion of rIgG[7]. Some rIgG preparations were absorbed 3 times with rabbit liver powder (40 mg rIgG 100 mg^{-1} liver powder ml^{-1}) to remove the cytophilic rIgG. Absorbed or not absorbed rIgG (20 mg ml^{-1}) was aggregated by heating at 63 °C for 20 min[2]. Human red blood cells (HRBC) (group 0) were coated with subagglutinating doses of rabbit IgG anti-HRBC (EA). HRBC were also coated with protein A of *Staphylococcus aureus* (ES)[8,9]. The cells were treated with 2 mg ligand 10^7 $cells^{-1}$ ml^{-1} for 30 min at 4 °C, afterwards washed 3 times with ice-cold TC-199 medium containing 2% bovine serum albumin. The cells were stained with appropriate amounts of FITC conjugates of anti-rabbit or human IgG or mixed with ES (5×10^7 ES 10^6 $cells^{-1}$ 0.5 ml^{-1}) and centrifuged at $100\times g$ for 10 min and further kept at 4 °C overnight[9]. The pellet was resuspended and at least 200 cells were counted. Cells with fluorescent membrane or cells with more than 4 ES were considered positive. The same technique was applied for EA rosette assay, but the cells were not treated with any ligand before rosetting.

Comparison of the binding of various rabbit IgG preparations to rabbit hepatocytes and macrophages as detected by rosette formation with human red blood cells coated with protein A of *Staphylococcus aureus* (ES)

Treatment	Percent of ES rosettes[a]	
	Hepatocytes	Macrophages
rIgG	46.8± 10.1	20.9±2.8
Heat aggregated rIgG	42.1± 12.1	36.0±6.0
rIgG absorbed[b]	8.3± 4.0	16.3±5.8
Heat aggregated rIgG absorbed	12.1± 5.4	24.7±9.3
Fc fragment from rIgG	17.9± 3.3	33.5±9.1
hIgG	21.7± 5.6	90.2±2.7
No treatment	1.3± 0.5	10.1±1.4[c]

[a] Mean of at least 4 separate experiments; [b] rIgG absorbed 3 times on liver powder; [c] Percent of macrophages bearing autologous cytophilic rIgG.

Results and discussions. The ability of various rIgG preparations to interact with rabbit hepatocytes was assayed by the ES-rosette technique, and the results of these experiments are illustrated in the table and figure 1. 3 prior absorptions of rIgG with liver powder almost abolished its binding abilities to hepatocytes (table). The amount of rIgG bound by the liver powder was less than 2% indicating that, of the total rIgG molecules, only a minor subpopulation possesses cytophilic affinity for the surface of rabbit hepatocytes. By heat-aggregation of non-cytophilic rIgG,

the molecule did not acquire a significant ability to attach to hepatocytes, proving that the aggregation of IgG molecules per se is not a condition for rIgG binding to hepatocytes. Moreover the lack of ability of HRBC coated with rabbit IgG antibodies (EA) to form rosettes with rabbit hepatocytes (3.7±1.8% EA rosettes as compared to 54.5±0.9% EA rosettes formed with macrophages) has confirmed the results of Hopf et al.[2] indicating that on the rabbit hepatocytes surface there are not FcR for antigen complexed IgG. The demonstration that FcR on liver cells are able to bind only monomeric IgG but not antigen complexed IgG, is in agreement with some recently reported results[13,14] showing that macrophage dispose of 2 distinct FcR, one for monomeric IgG and the other for antigen complexed IgG. It is possible therefore that on hepatocytes only 1 type of FcR is expressed. Immunofluorescent staining of cells charged with monomeric or aggregated IgG has shown that the aggregated ligand was much better visualized on the cell surface, due to the higher intensity of fluorescence. The binding of heat-aggregated IgG to hepatocyte surface is mediated by the cytophilic IgG molecules included in the aggregates, whereas the better UV-visualization on the cell surface of aggregated IgG than of monomeric IgG, showed by Hopf et al.[2] and confirmed by us in this work, is entirely due to the molecular size of the ligands. A model of the binding of IgG aggregates through their cytophilic IgG to hepatocytes and the consequences of the molecular size of the aggregates on the ability of immunofluorescence staining to detect their presence on the cell surface is illustrated in figure 2. This model explains why the presence of monomeric cytophilic IgG on cell surface could not be properly detected by fluorescence staining due to the low amount of cell-bound ligand. When the ES rosette method was applied, the presence of rIgG on hepatocytes was easily visualized due to the binding of ES to monomeric cytophilic IgG as well as to aggregated IgG. The sensitivity of the ES rosette method permits the detection of 10^4 IgG molecules per cell[10].

The percentage of rabbit hepatocytes binding hIgG was significantly lower than that binding rIgG (21.7% as against 46.8%). This behaviour is in contrast with that of rabbit macrophages, known to bind the heterologous IgG better than homologous one[11] (90.2% as against 20.9%). Since our experiments were performed in an allogenic system, the binding of rIgG as antibodies to rabbit hepatocytes had to be eliminated. Pretreatment of cells with Fab fragment failed to inhibit the binding of rIgG even at a 5 times higher Fab concentration, indicating that the interaction of rIgG with FcR hepatocytes was mediated by their Fc region. However, the binding of Fc fragment of rIgG to the surface of hepatocytes was diminished (table), suggesting that an intact hinge region may be necessary for the fixation of rIgG to FcR bearing hepatocytes. The in vivo induced immune complex deposition on hepatocytes, demonstrated by Hopf et al.[2], may be explained either by their binding through complement receptors (C3), shown to be present on the hepatocyte surface[2], or by the cytophilic IgG molecules randomly included in the antigen-antibody complex. The FcR on liver cell membrane functions as a binding site only for cytophilic IgG (less than 2% of the total IgG) conferring upon these cells the ability to remove these 'wornout' molecules from circulation[12]. In this way, the hepatocytes would be able to select for catabolism the cytophilic IgG from the native one, based on the affinity of their FcR for cytophilic IgG but not for native or aggregated (opsonic) IgG.

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On the contribution of haemolymph to the salivary proteins of the red cotton bug, *Dysdercus koenigii* F. (Heteroptera, Pyrrhocoridae)[1]

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Summary. Polyacrylamide gel electrophoresis of haemolymph and the salivary gland of adult male *Dysdercus koenigii* has been carried out. The presence of common bands in the electropherograms of the salivary gland and haemolymph shows the possible elaboration of haemolymph protein into the salivary glands as also evidenced by leucomethylene blue incorporation into the salivary gland lobules. The results are discussed in the light of our present state of knowledge.

Hitherto it was believed that the salivary lobules synthesize all the components of saliva. A recent review by Miles[2] shows that some of the salivary components are synthesized in the haemolymph and transported into the salivary glands[3,4]. The insect haemolymph has been the subject of extensive study since long[5-9]. Among the various haemolymph proteins, the female specific protein vitellogenin is well-known which is selectively sequestered by the developing oocytes[10-14]. However, the functions of other haemolymph proteins remain unclear. Therefore, the present study was undertaken with a view to finding out the contribution of non-vitellogenic proteins in the origin of saliva.

Materials and methods. Adult laboratory cultured males of *Dysdercus koenigii* were used in the present study. About 10 µl of haemolymph was collected from the amputated