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

antennae and dissolved in 0.2 ml of the sample buffer (0.02 ml 0.1 M PO_4 buffer, pH 7.5 and 0.18 ml 1% SDS and 1% 2-mercaptoethanol). 5 pairs of the salivary glands were dissected out from the animals whose haemolymph had already been taken out. The salivary glands were homogenized in 0.2 ml of the sample buffer. The disc gel electrophoresis was performed according to the method of Davis[15]. The haemolymph and salivary glands homogenates were centrifuged at 10,000 rpm for 15 min. The supernatants of these samples were treated at 100 °C in a water-bath for 3 min; 0.1 ml of each samples were loaded on 7.5% acrylamide gel containing 0.2% SDS. The gels were run in 0.1 M Tris-glycine buffer, pH 8.5 containing 0.1% SDS. Electrophoresis was conducted at a current of 3 mA/tube with a running time of 2 h. After the electrophoretic run, the gels were fixed in 50% TCA over-night at 4 °C and thereafter the gels were stained with coomassie blue for 2 h at room temperature. The gels were destained in a mixture of 50% methanol and 9.2% glacial acetic acid for 48 h and stored in 7% acetic acid. Leucomethylene blue[16] was injected into the haemocoel of the animals and incubated for 1 h. Whole preparations of the salivary glands were made.

Observations. Haemolymph. The electrophoresis reveals the presence of 15 protein bands of different thickness and intensities. Among these bands, there are 6 clear major bands (thick) which are numbered as 1–6 from the starting point (−) of the gel. Whereas there are 9 minor bands (thin) numbered as 7–15 (figure 1, H). Among the major bands, Nos 2–6 are more negatively charged and move further away towards the positive end of the gel, whereas band No. 1 is less negatively charged and moves little from the origin. However, the minor bands are less negatively charged and lie inbetween the origin and band No. 2. Among the minor bands, Nos 7, 11, 12, and 14 are faint (figure 1, H). Salivary gland. The electrophoresis results altogether in 10 protein zones (figure 1, S). Protein band Nos 1–5 are the major bands, while Nos 6–10 are the minor bands. These minor bands are more positively charged, similar to the minor bands of haemolymph, and remain confined below the major bands which are more negatively charged. A whole preparation of the salivary glands shows the incorporation of colloidal dye into the gland lobules in the form of various droplets (figure 2).

Discussion. The SDS polyacrylamide gel electrophoresis of the adult male haemolymph results in 15 protein bands, which are age-dependent[17], and there are 10 protein zones in the salivary gland. The comparison of the electropherograms of haemolymph and the salivary gland shows that 3 bands are common to both (figure 1, arrows). The lesser intensity of the protein bands in the electropherograms of salivary glands as compared to those of haemolymph might be the function of the quantity of the proteins which are being incorporated from haemolymph. Whereas the rest of the protein bands could be the gland cell's own products. The study with leucomethylene blue (vital dye) shows the

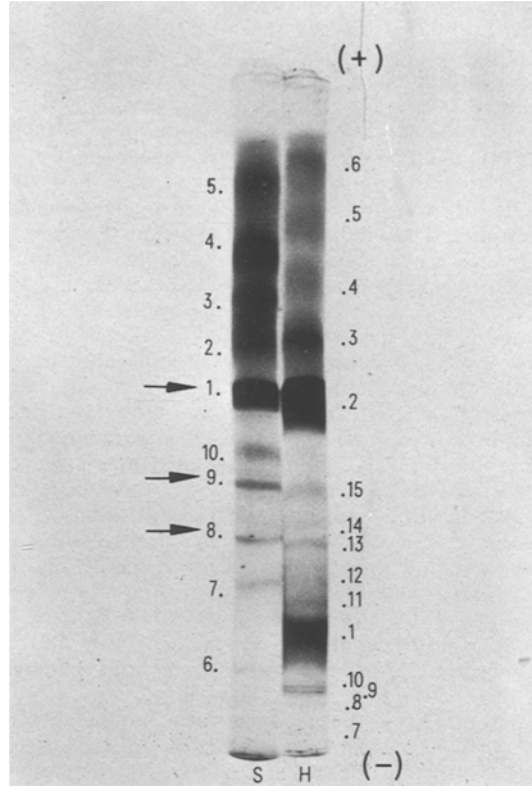

Fig. 1. SDS-disc gel electrophoretic separation of haemolymph (H) and salivary gland (S) from adult male *Dysdercus koenigii*. The haemolymph shows 15 protein bands and the salivary gland shows 10 protein bands. The common bands in the electropherograms (→) show the possible elaboration of haemolymph protein into the salivary gland. Electrophoresis was performed in 7.5% polyacrylamide in the presence of 0.2% SDS at pH 8.5 and the protein zones were stained with coomassie blue; gel diameter 6 mm.

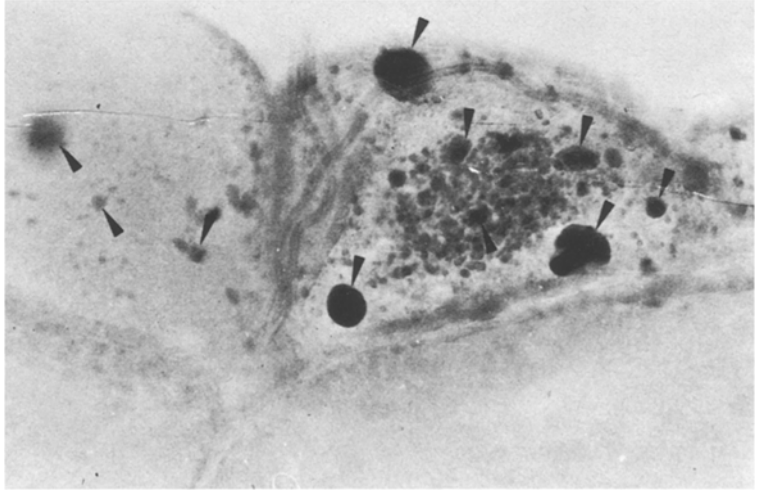

Fig. 2. Whole preparation of the gland, showing the incorporation of leucomethylene blue (→) in the gland lobules.

incorporation of this colloidal dye into some of the gland lobules as a function of haemolymph protein transport (figure 2). Therefore, the above studies give some evidence of haemolymph protein incorporation by the salivary glands[4], as has been established in the case of vitellogenin incorporation by the developing oocytes[18,19]. The present studies suggest that some haemolymph protein fraction is sequestered by the salivary glands, thereby resulting in heterosynthetic saliva. However, it awaits further immunological study.

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Effects of increased potassium in scala tympani on auditory nerve sensitivity

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Summary. Raising the K^+ concentration in scala tympani of the guinea-pig cochlea generally caused a substantial increase in the spontaneous firing rate of single auditory nerve fibres. This effect was not accompanied by any observed reduction in the threshold sensitivity of these fibres. These findings cast doubt on current theories of cochlear transduction.

The mechanical event of direct significance to the cochlear receptor mechanism is believed to be deformation of the hairs of the cochlear hair cell. According to the widely held Davis theory of mechano-electrical transduction[3-5] this deformation causes a resistance change in the hair cell membrane, accompanied by a current flow from the positively polarized endolymphatic space into the negatively polarized interior of the hair cell. Thus 2 biological batteries coupled in series provide a store of energy which can be tapped by mechanical events at the hair cell, and the threshold sensitivity of the cochlea derives from the large total polarization provided by these batteries across the hair bearing surface of the hair cell. The batteries give rise to the endocochlear potential (EP) and to the hair cell resting potential, respectively. The current flow will probably be carried mainly by K^+ ions since these are predominant in the endolymph that bathes the hairs and since the effective driving force through the hair cell is greater for K^+ than for the other main ion species in endolymph[4]. Support for the Davis theory has come from reports that agents which depress the EP simultaneously depress the auditory nerve gross action potential and substantially raise the minimum thresholds of primary auditory neurones[7-13]. The degree of dependence of threshold sensitivity in the auditory nerve upon the EP can be estimated from the work of Manley and Robertson[7]. These authors recorded from cochlear ganglion cells and reported that transient hypoxia which induced a reversible drop of 30 mV in the EP simultaneously produced reversible losses of about 60 dB in the minimum thresholds of cochlear neurones.

Because reductions in the EP produce substantial changes in auditory nerve sensitivity, it is to be expected from the Davis theory that reductions in the hair cell membrane potential would have a corresponding effect. We sought to test this by raising the K^+ concentration in scala tympani in order to depolarise the hair cells and to reduce the K^+ gradient between endolymph and perilymph, whilst simultaneously recording from single auditory nerve fibres.

Material and methods. Guinea-pigs were anaesthetised with urethane. The cochlea was exposed ventrolaterally and holes of 50–80 µm diameter were drilled into scala tympani of the basal turn at 2 mm from the round window, and at the helicotrema. A glass micropipette whose tip was broken to fit tightly into the basal hole served to introduce perfusates into the cochlea. This pipette was coupled to a microinfusion pump, which was used to perfuse at rates of 8–14 µl/min over about 1 min. Perfusates were modifications of an artificial perilymph described by Konishi and Kelsey[15]: the K^+ content was raised at the expense of Na^+ to maintain isotonicity. Various K^+ concentrations between 20 and 30 mM were used, most frequently 27.5 mM K^+ and 22.5 mM K^+. Recordings were made from the auditory nerve using glass microelectrodes filled with 4 M NaCl. Units were identified as primary by standard electrophysiological criteria[16].

Once a unit was isolated, elements of its response characteristics were determined manually, and thereafter data were collected with on-line use of a PDP-9 computer. In some units the response to a repeated tone pip at the characteristic frequency (CF) and the discharge in the 'silent' intervals between tone pips were studied. In other units rate-intensity functions for stimulation at the CF were sequentially plotted. For this latter procedure the unit was stimulated by 1-sec tone pips separated by 1-sec silent intervals: the discharge in each successive sec was counted and stored for as long as the unit was held, usually between 5 and 10 min. Starting at a chosen maximum the tone intensity was reduced by 6 dB between pips for each of 10 tone pips, whereupon this cycle was restarted.