H. virescens or *H. zea* are equally attractive to male *H. zea*. The Z-11-HDOL treatment caused a significant reduction in intraspecific mating of *H. zea* but not of *H. virescens*. Permeation of the atmosphere with Z-11-HDOL also caused interspecific attraction of male *H. zea* to the pheromone plume of female *H. virescens* and resulted in significant numbers of fatal matings between these individuals. However, the incidence of interspecific mating, was significantly lower than in the Z-9-TDAL treatment.

The 2 compounds (Z-9-TDAL and Z-11-HDOL) were subsequently tested in a 3-m long horse-show shaped wind tunnel made from clear poly-carbonate plastic with a floor of clear Plexiglass®. Air from 2 air conditioner units was blown by a variable speed fan through a Varicel® air filter and cheese cloth screens into the tunnel at a velocity of 50 cm/sec. Light from red colored light bulbs provided an illumination of 2.5 lux. Temperature in the tunnel was maintained at 25 ± 2 °C. Plume width as visualized by smoke from TiCl/4 was about 1 cm at the point of origin and 15 cm at the down-wind end of the tunnel. 20 µl of the synthetic mixtures of the pheromones of *H. zea* (11.5 ng of Z-11-HDAL, 0.45 ng Z-9-HDAL, 0.26 ng of Z-7-HDAL and 1.1 ng of HDAL/µl) or *H. virescens* (same amounts of the compounds as in *H. zea* pheromone plus 0.69 ng Z-9-TDAL, 0.23 ng TDAL and 0.9 ng Z-11-HDOL/µl) were applied to filter paper strips and suspended at 22 cm from the up-wind end of the tunnel. For air permeation 10 µg of the pheromone component was applied to a cotton dental plug and placed behind the blower fan. 2–3-day-old *H. zea* males were released singly from cylindrical cages at the down-wind end and their responses were monitored for 2 min. Five observations were made for each treatment and the test was replicated 14 times.

Results of these tests are given in table 2. Consistent with the field results, permeation of air with Z-9-TDAL caused a significant increase in the number of *H. zea* males that followed the *H. virescens* synthetic pheromone plume. The compound also significantly increased the number of males exhibiting hair-pencil extension (a pre-copulatory behavior) and attempts to mate with the pheromone source. However, permeation with Z-11-HDOL did not cause significant increase in the above responses.

Z-9-TDAL, and possibly Z-11-HDOL, are critical components in the *H. virescens* pheromone that are responsible for pheromone specificity and reproductive isolation. These compounds must permit *H. zea* males to discriminate between *H. zea* and *H. virescens* females in nature. We postulate that habituation to Z-9-TDAL or Z-11-HDOL affects this ability of *H. zea* males to discriminate. Furthermore, when the atmosphere was permeated with Z-9-TDAL, *H. zea* males even appeared to prefer *H. virescens* females to those of their own species, as reflected by higher trap catches.

The occurrence of interspecific matings has only been observed with tethered females and in laboratory cages. The importance of pheromone-induced fatal matings between these 2 species can only be determined after wide area field trials.

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A simple method of obtaining multiple blood samples from the portal vein and the hepatic vein in the rat in vivo

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Summary. A very simple and rapid technique for inserting a catheter in the portal vein and the hepatic vein in the anesthesized rat in vivo is described. The pointed, saline-containing PE tubing is frozen in liquid nitrogen, whereupon it is used as a 'needle' to insert the catheter into the blood vessel. Multiple blood samples can be obtained from the portal and the hepatic vein at the same time, so that in situ extraction of drugs by the liver can be measured in vivo, since hepatic blood flow is uninterrupted.

Recently a simple and rapid technique for obtaining serial blood samples from the portal vein in the rat has been described[1]. A catheter is inserted into the portal vein of the rat under anesthesia, after first inserting a 23-gauge needle in the vessel; the needle is slightly lifted ventrally to act as a guide for the polyethylene (PE) tubing used for catheterization. When the needle is removed, the catheter is fixed into place by a drop of cyano-acrylate ester cement.

Although this method is an improvement over previous methods, the portal vein still has to be teased free from the mesentery and there is some blood loss. Furthermore, the method cannot be used to catheterize the hepatic vein. The latter would be a very useful technique, because then the extraction of drugs by the liver in vivo could be followed. We present a simplified method for catheterizing both the portal vein and the hepatic vein while leaving the hepatic

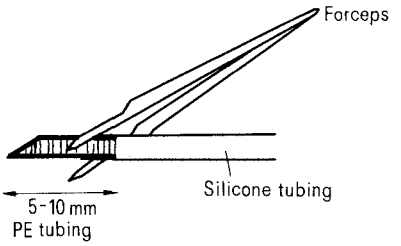

Figure 1. Catheter used for catheterization of the hepatic vein.

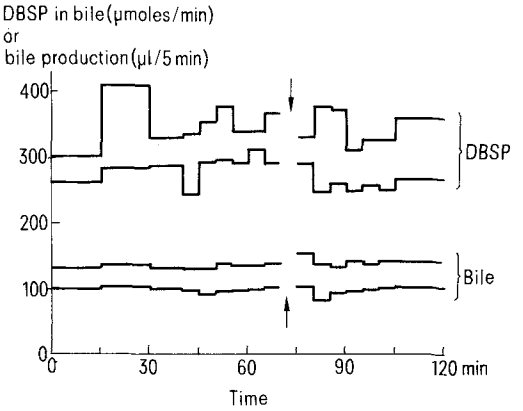

Figure 2. Effect of catheterization of the portal vein on bile production and biliary excretion of DBSP in the rat. Two male rats (300 g b.wt) were anesthesized with pentobarbital, and bile duct and jugular vein were cannulated as described before[4]. An infusion of DBSP was given in the jugular vein at a rate of 50 μmoles/kg/h, after a priming dose of 24 μmoles/kg. Bile samples were collected as indicated in the figure and DBSP in bile was determined[5]. At the arrow the catheter was inserted in the portal vein.

blood flow uninterrupted. The essential feature of the technique is that the PE tubing itself is used as the needle for insertion of the catheter in the blood vessel, by freezing it with liquid nitrogen.

PE tubing (outer diameter 0.8 mm, inner diameter 0.4 mm)[2] is filled with saline (or any other physiological solution), and one end of the tubing is cut at an angle of $50 \pm 5°$ so that it is pointed but not too sharp. The pointed end is put in a container of liquid nitrogen till the end is completely frozen. Meanwhile in the anesthesized rat the portal vein is exposed after laparotomy. Only the overlying thin membrane of the portal vein is cut away; otherwise the portal vein is left untouched. Using a forceps to hold it, the PE tubing is then inserted with the pointed, frozen end into the vessel, as if it were a needle. This should succeed at the first or second attempt; if it takes more time, the catheter has to be frozen again. Very little blood escapes during this procedure, even when the catheter is gently removed again. The PE tubing is fixed in position by a drop of cyanoacrylate glue. Blood samples can be taken, or an infusion can be given through this catheter. The whole operation, from cutting through the muscle wall and fixing the catheter, to closing the abdomen again does not take more than 2–5 min for a skilled technician, if assisted by someone who applies the glue to the catheter to fix it in position. The success rate is better than 90%, both for the portal vein and the hepatic vein. Occasionally, serious bleeding occurs and the rat has to be sacrificed. PE tubing with a smaller diameter cannot easily be used because it is not stiff enough when frozen. Also, teflon tubing, which is more inflexible than PE tubing, is not suitable because it is too smooth: it is almost impossible to penetrate the vessel wall.

For catheterization of the hepatic vein a slight modification is required. A 5–10-mm piece of the PE tubing is inserted into flexible silicone tubing[3] of a diameter such that no glue is required (fig. 1), to serve as a 'needle'. Flexible silicone tubing is used in order to accomodate the tubing in the limited space between the hepatic vein side of the liver and the diaphragm. The liver is completely exposed, and the membrane that connects the liver with the diaphragm is cut all the way to the bottom, to expose the vena cava and the hepatic vein. The diaphragm should not be damaged because that will stop breathing almost immediately. The hepatic vein is only very short; it is the confluence of the veins from the different lobes of the liver. After a very short common vessel, it joins the vena cava. When the vena cava and the hepatic vein are exposed the hepatic vein is located exactly, and the frozen tip of the catheter is inserted in this vessel, pointing towards the liver, using a forceps to hold the tip. This should be done very carefully so that the tip does not penetrate the other side of the vessel. When the catheter is in position some blood is withdrawn from the vessel to make sure that the catheter is indeed inside the vessel. Then a drop of the cyanoacrylate glue is used to fix the catheter in position in the hepatic vein. The flexible tubing is accomodated in the space above the liver, and glued to the rib cage with a drop of the glue. After this operation the abdominal incisions are clamped shut very carefully. Obviously, it is also possible to have catheters in the bile duct and the bladder[4] at the same time to collect bile and urine. Furthermore, we have catheterized the carotid artery and the jugular vein, to be able to take arterial blood samples as well, and to give an infusion.

Figure 2 shows the effect of catheterization of the portal vein on bile production and the biliary excretion of dibromosulphophthalein (DBSP)[5] in rats under pentobarbital anethesia. As can be seen, the bile production is hardly affected by catheterization. There is a very limited effect of catheterization on DBSP excretion in bile under steady state conditions: 10% or less. In agreement with the report by Urban and Zingery[1], we find that catheterization of the portal vein causes little disturbance in liver function.

This simple technique offers the unique possibility of measuring extraction of drugs across the liver in situ under conditions in which the circulation of blood through the liver is very little (if at all) affected by the surgical procedures. Possible effects of the anesthetic used during surgery obviously cannot be avoided, however.

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