Splanchnic and systemic hemodynamic data in five parabiotic pairs

	PVL (mean ± SEM)	Normal
CI ml·min^{-1}·kg^{-1}	413 ± 26*	318 ± 23
MAP mm Hg	117 ± 10	140 ± 6
PP mm Hg	15.4 ± 1.2*	11.1 ± 1.1
PSS %	95.9 ± 4.1**	0.3 ± 0.2
PVI ml·min^{-1}·100 g b.wt^{-1}	9.61 ± 1.29*	6.33 ± 0.36

CI, cardiac index; MAP, mean arterial pressure; PP, portal pressure; PSS, portal systemic shunting; PVI, portal venous inflow. * $p < 0.05$; ** $p < 0.001$.

predictable period of time (less than 2 weeks)[3]. Similar results were obtained in all the portal vein constricted mates of the parabiotic pair. However, a hyperdynamic circulatory state was not found in the normal parabiotic partners. In all the parabiotic pairs, plasma exchange was evident and the rate of exchange suggests that within several hours complete mixing of the plasma between the two animals takes place.

The results of the study do not support the existence of a transferable humoral factor mediating the hyperdynamic syndrome. On the other hand the existence of such an agent cannot be completely excluded by our findings. Benoit et al.[2] found a decrease in the intestinal arteriolar resistance of an isolated intestinal preparation of a normal rat when the preparation was perfused with blood from a portal hypertensive rat. It is possible that in the parabiotic rats despite a significant plasma exchange, the plasma levels of vasoactive agents sufficient to produce a hyperdynamic state may not be achieved because of dilution of these factors or their rapid metabolization by the liver of the normal rat. In the portal hypertensive rats most of the portal blood is diverted from the liver by the extensive portal systemic shunting. Although there is some increase in the hepatic arterial flow, the total hepatic blood flow is severely reduced[3]. In the normal rats, since the hepatic blood flow is unaltered the hypothetical vasoactive agents could be rapidly metabolized. The hyperdynamic syndrome could also be mediated by a change in receptor response which may further explain the lack of hyperdynamic changes in the normal rats.

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The inner ear structures of the echidna – an SEM study

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Summary. Surface structures of the echidna cochlea were investigated using a scanning electron microscope technique. It was found that unlike typical mammalian cochleas, the echidna cochlea possesses four rows of inner hair cells and 6–9 rows of outer hair cells, and that the arrangements of the stereocilia of the outer hair cells were not uniform throughout the length of the basilar membrane.
Key words. Echidna; cochlea; inner hair cells; outer hair cells; basilar membrane; scanning electron microscopy.

Monotremes are unique animals in that they possess some reptilian and some mammalian anatomical characteristics. There are three extant species: the platypus, *Ornithorhyncus anatinus,* the echidna, *Tachyglossus aculeatus* and the long-beaked echidna, *Zaglossus bruijnii*[2]. Available data suggest that the overall structure of the inner ear of the echidna, *Tachyglossus,* is closest in structure to that of other mammals. There are some interesting dissimilarities, however, such as the numbers of rows of inner and outer hair cells present on the basilar membrane[3,4]. A scanning electron microscope (SEM), which has been used extensively in investigation of mammalian cochlear surface structures, was used to clarify further the surface structures of the inner ear of *Tachyglossus.* It is possible to conclude from the present results that although it is probably most similar to that of other mammals, the echidna inner ear has several features characteristic of birds and reptiles.

Examination of available literature indicates that the mammalian cochlea has the following main characteristics: a) It is generally in a spiral of varying turns and widths, depending on the species examined[5]. b) It has three main fluid-filled canals (the scala vestibuli, the scala media and the scala tympani) with well defined round and oval windows at the basal end[6]. c) There are two types of hair cells; one row of inner hair cells (IHCs) and usually three rows of outer hair cells (OHCs) separated by a tunnel rod[7,8]. Exceptions to this are often found in humans and monkeys which frequently have four or five rows of OHCs at the apical turn of the cochlea[7]. d) Mature hair cells lack a true kinocilium[8]. e) Stereocilia of the OHCs are usually arranged in a 'W' shape[9] or a 'V' shape[10].

The similarities and dissimilarities of the echidna's cochlear structures to those of other orders of mammals can be summarized as follows: The cochlear duct of the echidna forms a banana-shaped curve rather than a spiral as in other orders of mammals, with a lagena at the distal end[8,11]. The cochlea has well defined oval and round windows, although the oval window is, in fact, round as is the case in birds and many reptiles[11]. There are the usual three fluid-filled canals within the cochlea (see fig. 1). Tunnel rods separate the inner and outer hair cells[8] (see fig. 2). There is a slight disagreement as to the number of rows of OHCs the IHCs on the basilar membrane. Previous studies[3,4] have found there to be three rows of IHCs and 4–6 rows of OHCs. Our results show that the numbers of rows of hair cells are quite different from those in other orders of mammals, and it is better to view the echidna as having four rows of IHCs and six

rows of OHCs to generally nine rows on the basilar membrane proximal to the lagena. The innermost row of the IHCs is more regularly and densely packed than the outer rows (fig. 2) and the arrangement of the stereocilia of each hair cell is less regular than that shown in other mammalian orders. In mammals, the stereocilia on the IHCs usually form 2–4 curved rows[12]; however, in the echidna they appear to show one or two rows (fig. 2). The rows of OHCs, however, follow a more uniform pattern. Only in the outer rows are the hair cells less regularly spaced (fig. 2). Although the cell bodies of the OHCs appear similar to those of other laboratory mammals, the arrangement of the stereocilia is not homogenous along the entire length of the basilar membrane. At the end proximal to the oval window, it alters; the stereocilia are in a dense, bushy arrangement similar to, but less uniform than that found in birds and reptiles (fig. 3). The configuration of the OHC stereocilia in other mammals also changes to a certain degree along the length of the cochlea. However, it is the angle of the 'arms' of the 'W' or 'V' which varies[9]. No evidence of a kinocilium was found in the specimens examined.

So it appears that the echidna cochlea is similar to that of other mammals in many respects, but is much less coiled, and the numbers of rows of inner and outer hair cells differ. The possession of a lagena and also the presence of some stereocilia in a dense, bushy arrangement proximal to the oval window is characteristic of birds and reptiles.

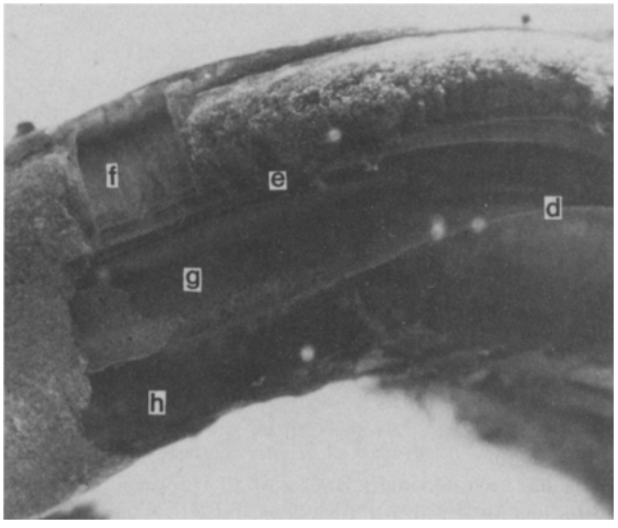

Figure 1. Showing the three well defined fluid-filled canals which are very similar to those found in other mammalian cochleas. d, Reissner's membrane; e, basilar membrane; f, scala tympani; g, scala media; h, scala vestibuli.

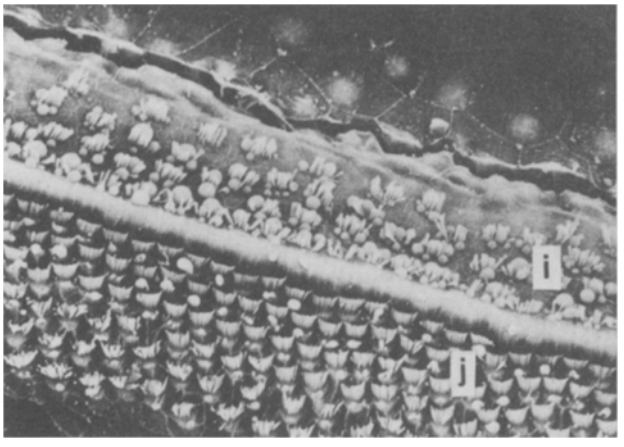

Figure 2. Showing four rows of inner hair cells and six rows of outer hair cells separated by a tunnel rod. i, 4 rows of IHC; j, 6 rows of OHC. Magnification: × 2700.

Figure 3. Showing the cell bodies and the 'bushy' arrangement of the stereocilia of the outer hair cells proximal to the oval window. The scale (2nd line top left corner) is equal to 1 μm.

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A study on the pharmacokinetics in mouse of adenine-9-β-D-arabinofuranoside 5-monophosphate conjugated with lactosaminated albumin[1,2]

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Summary. In plasma of mice injected with adenine-9-β-D-arabinofuranoside monophosphate (ara-AMP) coupled to human lactosaminated serum albumin (L-HSA) some of the ara-AMP molecules are enzymatically released, whereas others remain linked to L-HSA. Evidence has been obtained that ara-AMP is not deaminated when it is conjugated to L-HSA, in contrast to the free drug which is rapidly metabolized to its hypoxanthine derivative.
Key words. Adenine arabinoside 5-monophosphate; drug targeting; hepatitis B.

In order to increase the chemotherapeutic index of adenine-9-β-D-arabinofuranoside monophosphate (ara-AMP) in the treatment of chronic hepatitis B, this drug was conjugated with human lactosaminated albumin (L-HSA)[3-6], a neoglycoprotein with terminal galactosyl groups which is taken up only into hepatocytes, where it is digested in lysosomes[7-9]. In mouse, L-HSA-ara-AMP conjugates enter liver cells almost exclusively; only very small quantities are taken up by the other organs[6]. Administered to mice with *Ectromelia* virus hepatitis they inhibit virus DNA synthesis in liver without significantly inhibiting cellular DNA synthesis in intestine and bone marrow[3-6]. L-SA-ara-AMP conjugates prepared with homologous L-SA are devoid of humoral and cellular immunogenicity at least in mice[10]. For details of abbreviations see under references[2].

In order to obtain some data on the pharmacokinetics of L-HSA-ara-AMP conjugates, in the present experiments we 1) developed a radioimmunoassay (RIA) enabling us to measure ara-AMP linked to L-HSA in mouse plasma; 2) studied the stability in vitro of the bond between ara-AMP and L-HSA in mouse and human plasma; 3) investigated the rate of disappearance of protein-bound radioactivity from plasma of mice injected with conjugates labeled in the protein or in the drug moiety.

Materials and methods. Lactose was coupled to HSA (crystallized, essentially globulin free) (Sigma) by reductive amination with $NaBH_3CN$[11,12]. The reaction was stopped when the molar ratio sugar/protein reached the value of 30 (L_{30}-HSA). Ara-AMP (Warner-Lambert) was conjugated with L_{30}-HSA by a slight modification of the method of Fiume et al.[13]. In different conjugate preparations the molar ratio ara-AMP/L-HSA, determined spectrophotometrically, ranged from 6.1 to 6.4; it is indicated by the number under the drug (e.g. L_{30}-HSA-ara-$AMP_{6.1}$). With the same procedure, hypoxanthine-9-β-D-arabinofuranoside 5-monophosphate (ara-HxMP) (ICN) was coupled to L_{30}-HSA. The resulting conjugate had a molar ratio ara-HxMP/protein of 4.7.

Three radioacitve conjugates, one labeled in the protein and two in the drug moiety, were prepared. The first one (L_{30}-[^{3}H]HSA-ara-$AMP_{6.2}$) was obtained by coupling ara-AMP to L_{30}-HSA which has been previously labeled with [^{3}H]formaldehyde (100 mCi/mole) (NEN) in the presence of $NaBH_3CN$[14] according to the procedure previously described[6]. The specific activity of the conjugate was 1.3×10^6 dpm/mg. To prepare the conjugates radioactive in the drug moiety, tritiated ara-AMP (either [2-^{3}H adenine] 22 Ci/mmole, ICN or [2,8-^{3}H adenine] 16 Ci/mmole, Amersham) was diluted with the cold drug and coupled with L_{30}-HSA. The resulting conjugates L_{30}-HSA-ara-[2-^{3}H]$AMP_{6.1}$ and L_{30}-HSA-ara-[2,8-^{3}H]$AMP_{6.4}$ had a sp.act. of 5.8×10^5 and 6.8×10^5 dpm/mg respectively.

In order to study the cleavage of the bond between ara-AMP and L-HSA in mouse or human plasma, in vitro, 180 or 750 μg L_{30}-HSA-ara[2,8-^{3}H]AMP in 50 μl of saline (NaCl 0.9%) were added to 950 μl of heparinized plasma or saline pre-heated to 37°C. After different times (at 37°C, with shaking) 40 μl of the mixture was gel filtered on a 1.5 × 5 cm Sephadex G-25 Medium

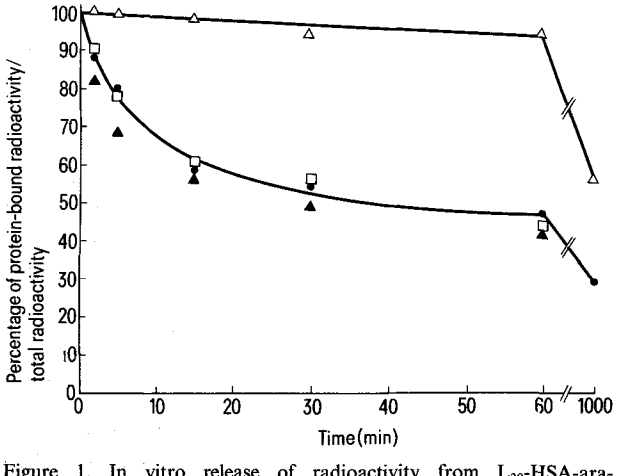

Figure 1. In vitro release of radioactivity from L_{30}-HSA-ara-[2,8^{3}H]$AMP_{6.4}$. △, in saline (750 μg conjugate/ml); ●, in mouse plasma (750 μg/ml); ▲, in mouse plasma (180 μg/ml); □, in human plasma (750 μg/ml).