centrifuge tube. The tube was gently rotated to mix both solutions thoroughly and centrifuged at 3,000 rpm for 10 min to obtain a clear supernatant. The optical density of the supernatant obtained from the dyed blood was read at 570 nm against the supernatant obtained from the undyed blood. Dyed plasma separated from the packed red blood cells was used for preparation of a standard solution of the dye. $^3/_{100}$ ml of the dyed plasma was pipetted into a 50 ml-graduated flask and diluted with Ringer solution. The optical density of the standard solution was read against Ringer solution. In the course of pipetting 0.03 ml of packed red blood cells into 2.0 ml of Ringer solution as mentioned above, the red blood cells adhering to the pipette were washed several times with the Ringer solution so that any visible trace of the red blood cells was transfused into the solution. This procedure was done very carefully in order to prevent hemolysis. If the supernatants were reddish, the results from these samples were discarded. Percent of plasma trapped in the packed red blood cells was calculated according to the method of JACKSON and NUTT[1]. Red blood cell counts were determined by a routine technique under a light microscope. Mean corpuscular volume (MCV) was calculated as red cell volume/red blood cell counts.

Results and discussion. The Table shows that, under our experimental conditions, the trapped plasma content in the packed red blood cells was 1.5% for maternal blood and 1.1% for fetal blood, the difference between these values being significant ($p < 0.02$ according to Student t-test). In ox blood, the percentage was markedly high, at 5.3%. The Table also indicates that the magnitude of trapped plasma is inversely related to MCV, i.e., the smaller the MCV, the higher the percentage of trapped plasma. This relationship supports the results of CHIEN et al.[2] in which percentages of trapped plasma were 1 to 3% in bloods of elephant, man and dog (MCV 112 to 72 μm^3), 4% in sheep blood (MCV 37 μm^3) and 9% in goat blood (MCV 18 μm^3) under the conditions of centrifugation at $15,000 \times g$ for 5 min. The MCV values for maternal, fetal and ox bloods obtained in our study were quite similar to those described elsewhere[3]. When erythrocytes of small diameters, such as those found in ruminants, are subjected to experiments such as hematocrit determination and concentration measurements using specimens of packed red blood cells, errors caused by intercellular plasma trapping should be considered in order to obtain more accurate results.

Percent of trapped plasma and mean corpuscular volume (MCV)

Blood	Trapped plasma (%)	MCV (μm^3)
Maternal	1.5 ± 0.10 (15)	96.5 ± 3.94 (6)
Fetal	1.1 ± 0.11 (13)	116.2 ± 5.16 (7)
Ox	5.3 ± 0.29 (15)	60.0 ± 3.34 (6)

The values represent mean ± SE. Numbers of samples measured are shown in parentheses.

Zusammenfassung. Blutproben verschiedener Herkunft enthielten nach 5 min Zentrifugation bei 15000 g im Erythrozytensediment folgenden Plasmagehalt: menschliches Erwachsenenblut 1,5%, menschliches Nabelschnurblut 1,1% und Ochsenblut 5,3%.

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Hyperacute Graft Rejection in *Eisenia foetida typica* and *Eisenia foetida unicolor*

The acceptance or rejection of grafted tissues and organs has been extensively studied in vertebrates and much knowledge has been gained of the underlying immunological mechanisms which lead to rejection of allografts and xenografts in immunologically competent recipients. In an attempt to study the evolutionary origins of these mechanisms, some preliminary investigations have been carried out in invertebrate systems, particularly in the earthworms where transplantations of body wall provides a convenient assay procedure.

Autografts, allografts and xenografts have been studied in *Eisenia foetida* and *Lumbricus terrestris* and the degree of rejection has been monitored by the loss of pigment from the graft surface[1-3]. Most autografts heal in successfully and are fully accepted; allografts are initially accepted but later undergo acute (2–20 days) or chronic (20–250 days) rejection; xenografts are usually rejected in an acute manner. Earthworms carrying a primary graft generally reject a second allograft or xenograft in an accelerated fashion similar to the 'second set rejection' of vertebrates and indicating immunological memory. It is also claimed[4] that accelerated rejection may be achieved in an unsensitized recipient by transferring coelomocytes from the putative donor.

In all the published reports there is mention of extremely rapid graft rejection occurring within 24 h. This rapid rejection is also seen in a proportion of autografts and has therefore been ascribed to 'technical failure' rather than incompatibility. From initial experiments, I gained the impression that this 'hyperacute' rejection was more often seen with allografts and xenografts than with autografts. The present investigation was designed to determine whether this was indeed so, and whether a proportion of graft rejections put down to technical failure might instead be related to incompatibility between donor and recipient.

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The earthworms used were *Eisenia foetida*, isolated from known locations and maintained in the laboratory for at least 2 years, and *Dendrobaena veneta* collected from the wild. The worms were cultured in a peat-chalk mixture (100:1), in petri dishes and maintained at 15 °C. Grafts were made between clitellate worms of similar age (120 days) and size (60 mm). The worms were anaesthetized for 5 min in 7% ethanol in earthworm saline and grafts cut from the dorsal body wall (3 × 2 mm) with fine scissors. The graft bed of the host was not swabbed out to remove excess coelomic fluid, the graft was placed in this maintaining its original posterior anterior orientation after some trimming if necessary to allow for a good fit. No sutures were used to hold the graft in place. Each operation was carried out whilst the animal was laid on a cold plate maintained at 2 °C. The worms were then transferred to individual petri dishes containing damp filter paper. The experimental material was observed at 2 hourly intervals for the first 6 h, after which time they were moistened again, they were then observed at 24 hourly intervals. Grafts were made between the donor-recipient combinations shown in the Table which also shows the percentage of hyperacute rejections for each combination. Autografts showed a hyperacute rejection rate of about 20% and as there should be full compatibility, this rate probably represents a true base level for technical failure. The hyperacute rejection rate for primary allograft and xenograft combinations is in all cases considerably greater and χ^2 analysis shows the differences to be significant at 0.05% level. With second set grafts there was marked hyperacute rejection when the time interval between first and second graft was 5 and 8 days. Published work suggests this time interval between first and second grafts produces the greatest accelerated rejection of second grafts[4].

Summary of graft combination and statistical analysis

Donor		Host	Hyperacute rejection (%)	χ^2
ET	→	EU	53	4.3796[a]
EU	→	EU	39	
EU	→	ET	55	7.7815[b]
ET	→	ET	35	
ET	→	ET	35	0.2556
EU	→	EU	20	
ET	→	EU	53	0.3070
EU	→	ET	55	
EU	Autograft		20	3.8788[a]
EU	→	EU	39	
ET	Autograft		24	1.5568
ET	→	ET	35	
EU	Autograft		20	10.2546[b]
ET	→	EU	53	
ET	Autograft		24	9.5085[b]
EU	→	EU	55	
EU	Autograft		20	7.2180[b]
DV	→	EU	52	
ET	Autograft		24	4.0614[b]
DV	→	ET	50	
EU	→	EU		
Same population				
5 day interval between grafts				
1st set graft			29	21.5666[b]
2nd set graft			50	
8 day interval between grafts				
1st set graft			28	4.4979[a]
2nd set graft			75	

EU, *Eisenia foetida unicolor*; ET, *Eisenia foetida typica*; DV, *Dendrobaena veneta*. [a] $0.05 > p > 0.01$. [b] $0.01 > p > 0.001$.

It seems likely then that this excess of hyperacute rejections seen in allografts and xenografts does reflect the incompatibility of donor and recipient. Graft rejection within 24 h in such combinations can no longer be ascribed solely to technical failure, and the underlying mechanisms, whether cellular or humoral, deserve further study.

Zusammenfassung. Das Phänomen der hyperakuten Transplantat-Abstossung (innerhalb von 24 h) wurde bei Invertebraten untersucht. Diese rasche Abstossungsreaktion wird signifikant häufiger bei Allo- oder Xeno- als bei Autotransplantationen beobachtet. Dies spricht für das Vorliegen einer Inkompatibilitätsreaktion.

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The Influence of Cyclic Nucleotides (c-AMP and c-GMP) on the Nitrous Blue Tetrazolium Reduction by Human Polymorphonuclear Leucocytes

The participation of Adenosine 3′:5′-cyclic monophosphate (cyclic-AMP) in various metabolic processes accompaning phagocytosis of particles by polymorphonuclear (PMN) leucocytes is the subject of controversy in recent literature[1–3]. SCHELL-FREDERICK[4], recently reported that addition of cyclic-AMP or related substances does not influence the glucose oxidation and CO_2 production, the two basic processes stimulated by particle ingestion. QUALITIONE et al.[5] demonstrated an inhibition of hexose-monophosphate shunt activity following administration of cyclic-AMP, dibutyryl cyclic-AMP and caffeine.

These contradictory reports prompted us to investigate the influence of cyclic-AMP and substances known to

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