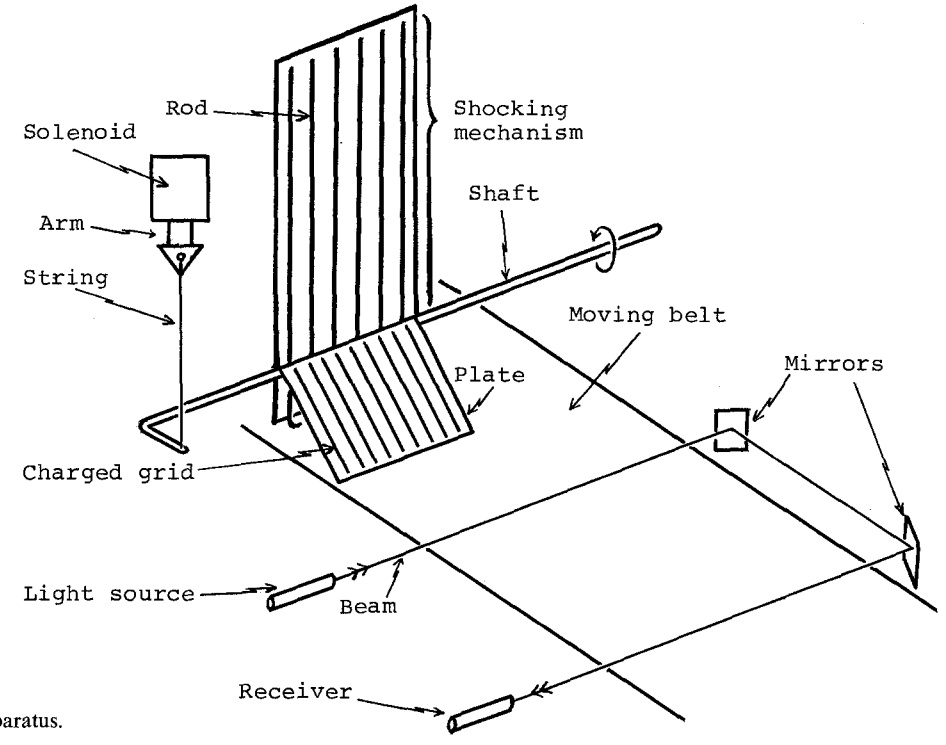


Figure 1. Diagram of improved apparatus.

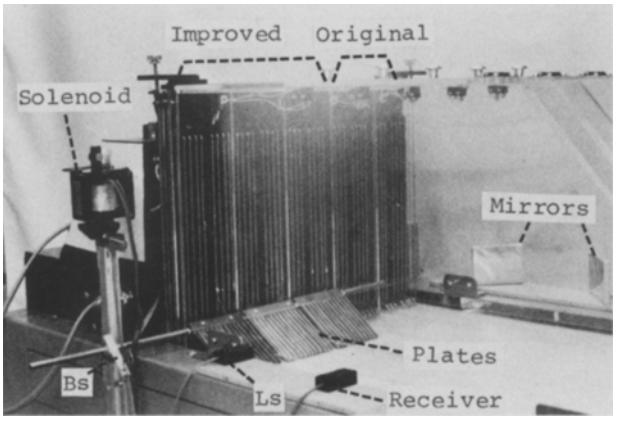


Figure 2. 2 original and 3 improved lanes are shown. The branch of a shaft (Bs) is connected with a string to the arm of a solenoid. Ls: Light source.

injuries, but also kept the surface of the belt clean. Please note the fact that short-circuits may occur frequently unless the surface of the plate is coated with wax.

The construction was easily achieved by the authors, and the total cost was less than 130 dollars.

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## A simple method of preparing chick eggs for chorio-allantoic grafts

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*Summary.* A method of preparing chick hosts for receiving grafts to the chorio-allantoic membrane (CAM) is described, which is quicker and requires less manipulative skill than that in general use.

The CAM has been used many times as a site for transplanted tissues since Rous and Murphy first reported its ability to support the growth of tumors[1]. The method almost invariably employed nowadays is the 'artificial air space' technique[2-6], in which a small hole is first made through the shell to the air space (as the egg lies with its long axis horizontal), and a square of shell is then cut from the dorsal surface, using a small file or power tool fitted with a fine carborundum disc. The underlying shell membrane is moistened and torn, and the contents of the egg then drop, expelling the air from the air space, leaving a cavity beneath the window with the vascularized CAM as its floor. However, this technique presents certain difficulties. The delicate vascularized membranes lying underneath the shell may be damaged during the windowing procedure, either directly by the instrument penetrating through to them, or by friction-generated heat. This, of course, can usually be avoided by taking care, but the procedure then becomes progressively slower to perform, which may be a real disadvantage if numbers of hosts are to be prepared. Damage to membranes may lead to subsequent death of the host.

Even after the graft has been made, it may not 'take' successfully on the surface of the CAM. The reasons for this are not clear, but methods for increasing grafting success often rely on bringing the graft and CAM into closer proximity[7,8], by placing the graft underneath a carrier material. It is possible that the layer of moisture which covers the CAM after the windowing procedure otherwise causes the graft to float, delaying the intimate contact necessary before neo-vascularisation can begin, and perhaps leading the graft to displace from its original site after the operation.

In view of the importance of the problems which are studied with the aid of this technique (e.g. tumor invasiveness[9,10]), any improvement which makes the technique quicker and easier to perform may be of interest. Such an improvement is described below.

*Materials and methods.* The eggs are incubated with the long axis vertical, and the air space (blunt end) up. After the desired period, they are removed from the incubator, and placed on plasticine egg rests in the same orientation as they were incubated. The egg is wiped with a tissue soaked in 70% ethanol as are all the instruments employed at each use, and a hole is made in the shell above the air space with coarse forceps. The air space may be easily seen if a strong light is directed at the egg. The hole is enlarged until convenient access can be gained to the floor of the air space, then the shell membrane is carefully pulled back using fine (watchmakers') forceps, to expose an area of CAM just slightly larger than the graft. The graft is placed on the CAM with fine forceps if this is possible; otherwise a pipette may be used but excess moisture should be removed with a sterile tissue. Then the egg is resealed with Sellotape or Scotch tape - 2 pieces may be necessary to make the seal air-tight - and the egg is returned to the incubator.

*Results.* Going through the air space in this way offers a number of advantages over the normal method: a) It is quicker and easier to perform, especially for those unfamiliar with the chick egg as host. b) It does not require that the membrane be moistened, and it is therefore likely that the graft will make immediate contact with the CAM. c) The area of CAM exposed is only slightly larger than the graft, and when the egg is re-opened for graft retrieval, the site of the operation is clearly identified. It is therefore immediately obvious where the grafts may be found: they do not translocate by this method.

It is not possible to quote general success rates by this method, as obviously they will vary from application to application. However, in 1 experiment carried out by this method, I grafted chick limb buds of stages 19-24[11] to the CAM of 7-day hosts, and examined the host eggs after a further 7 days incubation. 52 of the 54 hosts employed survived this period, and from these, 47 grafts which had vascularized and developed further were retrieved, i.e. the grafts were successful in 90.4% of the survivors. This compares well with conventional methods.

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