TABLE 1

PROTECTION OF HUMAN PLASMA AGAINST THE EFFECT OF HYALURONIDASE BY DIFFERENT CONCENTRATIONS OF PLATELETS*

Amount of platelets (in mm^3 of added suspension)	0	1×10^5	2.5×10^5	5×10^5	2×10^6
Clotting time of platelet-plasma mixture	177 sec	94 sec	85 sec	85 sec	82 sec
Clotting time of platelets-hyaluronidase-plasma mixture	∞	$>$ 1000 "	820 "	450 "	197 "
Prothrombin time of platelets-hyaluronidase-plasma mixture (after addition of 0.2 ml brain thromboplastin)	10.1 sec	10.2 "			10.1 "

* Five tenths ml of platelet suspension incubated for 30 min at 38° C with 1 mg dialyzed bovine hyaluronidase. After incubation, 0.5 ml human oxalated, dialyzed plasma was added to each sample and incubated for 10 min. Recalcified with 0.5 ml $CaCl_2$ (0.02 *M*), pH 7.5.

hyaluronidase, their clot-accelerating activity was overcome and the enzyme retained less activity (or none) against the plasma (Table 1). The enzyme, counteracted by an excess of tissue (brain) thromboplastin, could inhibit the diluted thromboplastin (1:100–1:1000), so that in respect to hyaluronidase inhibition the same factor appears present in both tissue and platelet thromboplastic component.

The enzyme after its incubation with plasma could not be recovered. There is, moreover, some evidence that the effect of clotting inhibition by even small amounts of hyaluronidase is not dependent on the incubation time. Thus it seems likely that, rather than providing a substrate in the conventional sense, the thromboplastic component both in plasma (from the platelet material still present) and in platelets interact with the enzyme competitively, with resulting inhibition of the plasma clotting mechanism and of hyaluronidase, one or the other inhibition being more in the foreground, depending upon the relative excess of either hyaluronidase or platelet material.

So far as platelets are concerned, it was found that these elements do not contain any measurable amount of nucleic acid (UV-spectra of sonically disrupted platelets) but, in addition to protein material absorbing at 280 mμ and inactive in coagulation, they do contain material that competes with hyaluronidase, withstands boiling for 15 min, and is not precipitated by 5% trichloracetic acid. This nonprotein material which does not pass through the cellophane dialysis membrane has the same accelerating effect on the coagulation as the intact platelets. The chemical nature of this thermostable, nonprotein thromboplastic material, which interacts with hyaluronidase in the same manner as described above, and which corresponds to what has been designated by Howell (*2*) as "cephalin" thromboplastic factor, is under investigation by means of paper chromatography. In connection with the inhibitory effect of hyaluronidase on thromboplastic blood component the further purification of enzyme preparations is needed. It may be recalled, also, that the thromboplastic effect of highly polymerized hyaluronic acid has been reported (*3*).

Although the concept of the interaction of platelets and a plasmatic factor in constituting the natural thromboplastin in blood coagulation is fully valid, the particular formulation of Quick (*4*), supposing the active principle in platelets to be an enzyme (thromboplastinogenase), is incompatible with the findings here reported, which exclude the protein nature of the thromboplastic platelet factor. A detailed study of the thromboplastic system operating in blood will appear shortly.

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A Tissue Chamber and Splint for the Mouse[1]

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Technical problems of splint and chamber construction encountered while engaged in tissue chamber studies of skin grafts in mice prompted some experimentation. A type of splint was devised which could be constructed from copper wire, sheet lucite, and solder with the aid of a few tools. It is offered to those who wish to undertake this fundamental mode of animal experimentation, but who have little machine-shop aid, and whose laboratory time is limited. It is well tolerated (4 weeks or longer) and satisfies the basic requirements of (1) adequate immobilization and protection, (2) maintenance of proper circulation within the chamber, and (3) provision for satisfactory microscopic observation. It has been used successfully in conducting several series of studies of skin grafts in mice.

Although the essential principles of construction described in detail by Algire and Legallais (*1*) have been followed, the skin flap is immobilized with a traction splint of copper wire, and the tissue chamber proper is incorporated within one of the lateral splints of lucite. The chamber itself is closely similar to the

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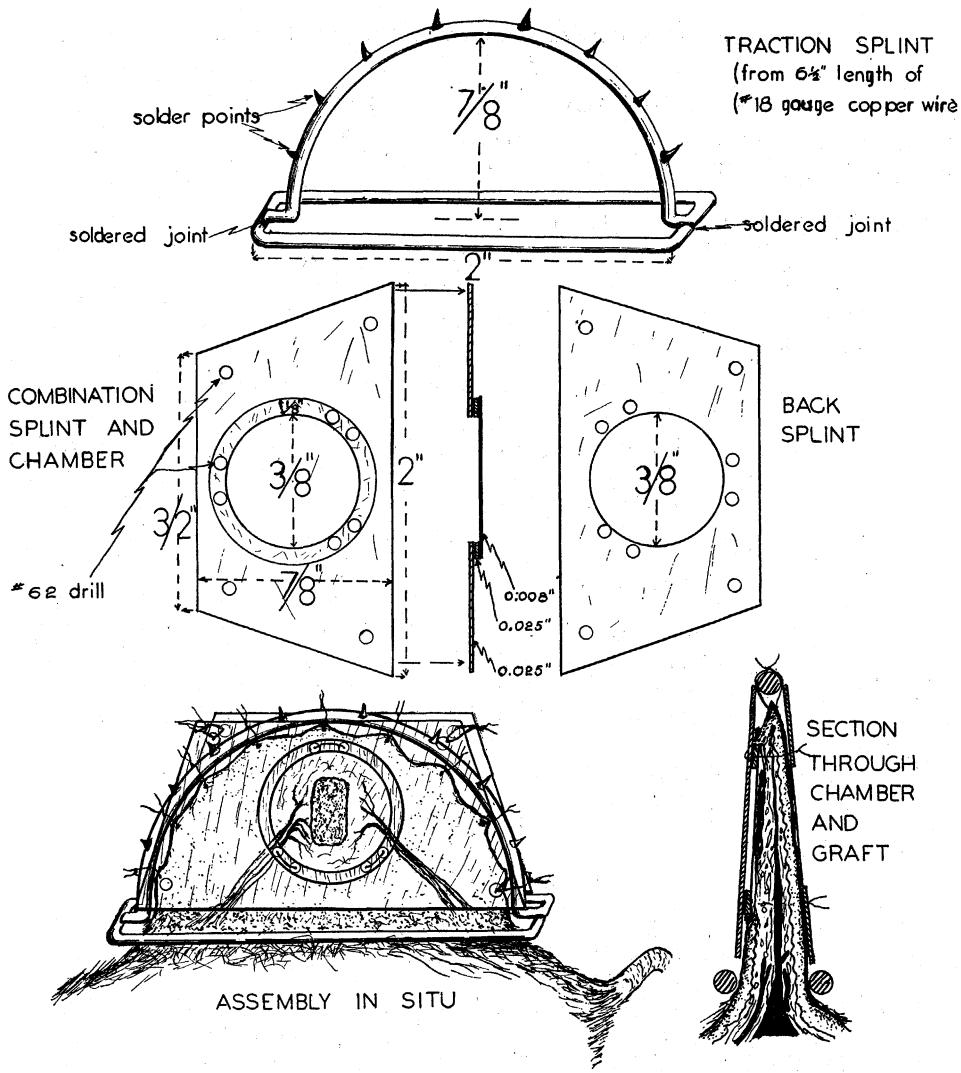

FIG. 1. Mouse tissue chamber.

"preformed tissue" type of Algire and Legallais. Trauma to the vessels of the exposed panniculus carnosus of graft bed, and dislodgment of the graft such as might occur during insertion of the separate chamber through an aperture in the splint, are avoided; splint and chamber are placed over the wound, the edges of which are then gently teased around it, without damage to either graft or bed.

Construction of splint and chamber. A traction splint of copper wire (18-gauge, bare) is made by bending a 6½" length around a wooden or lucite semicircular block of dimensions as shown (Fig. 1), which is grooved shallowly to hold the wire in position. The splint is soldered at the places indicated. Drops of solder are applied to the well-burnished wire ("flowed on") and then pulled out to points by touching them with the iron as it cools. The points prevent slipping of the tantalum wire (0.003") traction sutures along the wire arch. Although no irritation from the metals has been observed to date, the wire may be sprayed, if desired, with clear lacquer.

Combination splint and chamber and the back splint are cut from sheet lucite (0.025"), the blanks stacked in piles of 10 or 12, and the suture holes drilled with a #62 drill. The circular apertures of both splints are cut out with a ⅜" wood gouge. The windows for the chamber are cut from thinner sheet lucite (0.008") with a punch made from brass tubing (⅝"). The supporting rings for the chamber are either made from a tube of lucite, turned and drilled on a lathe to size and rings cut therefrom with the small circular saw of the drill kit, or are cut out with gouge and punch from 0.025" sheet lucite.

Splint, ring, and window are then cemented together with lucite cement (5% solution of lucite in chloroform) to form the combination splint and chamber, and placed under pressure for several hours to insure firm union. The 6 holes for the chamber sutures are

then drilled (#62 drill or smaller, depending upon size of needle to be used). All sharp edges around the chamber are removed with a fine file or sandpaper.

The lower figures of the illustration show the lateral and cross-sectional views of splints and chamber in position on the mouse, the graft resting on the panniculus carnosus beneath the transparent chamber.

Operative procedure: Time is saved if anesthesia is induced with ether, immediately supplemented with 0.2 ml (1.2 mg) of a 1:10 dilution of Veterinary Nembutal. Clipping and depilation (with depilatory cream) may then be done without delay. A wooden or lucite block (3″ × 2″) provided with a V-groove is used to hold the mouse in position during passage of sutures, skin dissection, and fixation of the chamber.

The four arteries of the skin of the dorsum being identified by transillumination, they are apposed, and the fold of skin transfixed with a suture at the highest point of the skin fold, or seized with towel forceps, which enclose the wire arch also, at this same point. Fixation sutures are then passed through the periphery of the skin fold, and fastened by twisting with serrafin clamp or by tying. If the very small arterial needles (#16 Diamond drill-eyed sharps) are not available, #9 Milliner's may be used. The base of the traction splint may be given additional anchorage by passing sutures through it and the skin, at its two ends.

Should loss of proper flap tension or loosening of sutures occur, additional or replacement sutures are readily placed where needed, with this type of splint.

The fold of skin being fixed in traction, graft bed and chamber site are prepared as follows: At the center of the proposed chamber site, the skin is carefully nicked with fine scissors and, by blunt dissection, gently raised in radial fashion from the underlying panniculus carnosus. This procedure is facilitated if an initial bleb of skin is raised by injection of a drop of saline with a very fine hypodermic needle. The separation of skin from panniculus completed, the skin is removed so as to leave a circular wound of approximately the same diameter as that of the chamber (*2*). During this procedure damage to the vascular panniculus must be scrupulously avoided. Only one skin layer is treated in this fashion, its panniculus being the bed of the graft. The opposite skin layer and panniculus are preserved intact.

Splint and chamber are then placed over the circular opening, and over the graft (if used). The back splint is then applied, and sutures are passed through both splints and around the wire traction splint, as shown. The three through-and-through sutures of the chamber are then passed and tied after making sure that the chamber is lying upon the panniculus, and within the edge of the skin wound. A seal of fibrin from the exudate soon cements the outer edge of the chamber to the edge of the circular incision.

Materials required are sheet lucite: 0.025″ and 0.008″; lucite cement: 5% lucite in chloroform; copper wire: 18-gauge, bare; solder: resin core.

Tools required are drill press: "Handee," "Casco," etc.; drill: #62 (64); circular saw blade for drill; soldering iron: "pistol type" preferred, with fine tip; pliers: one long-nosed, one side-cutting; bench-vise: small; shears or heavy scissors; wood gouges: ⅜″ and ⅝″; brass tubing: ⅝″ ID; small files; fine sandpaper.

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A Variable Heart Pump Permitting Independent Control of Rate, Output, and Ejection Velocity

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Since the cardiovascular apparatus is an integrated system, it is often difficult to differentiate between purely cardiac and purely vascular responses. One approach to the understanding of the functioning of a complex system is to isolate its various parts. In this way the responses of each segment may be studied under controlled conditions undistorted by the reactions of the other parts. A great advance was made by Starling (*1*) who, utilizing the living heart and lungs, substituted an artificial vascular system which could be altered at will. Starling thus was able to analyze the effects on the heart alone of controlled variations in venous return, temperature, and peripheral resistance. However, the reverse experiment, that of studying the reactions of the isolated vascular system in response to controlled variations in the cardiac pumping mechanism, has not yet been undertaken.

Recent advances, particularly the development of plastics which do not interfere with the coagulability of the blood, have made it possible to construct a workable "artificial heart" (*2, 3*). It seems possible, therefore, to develop a heart pump that can be altered at will over a wide range in regard to rate, output, and ejectile velocity and to substitute this pump for the living heart in the experimental animal. A means would thus be provided for studying the effects of controlled variations in the cardiac pumping mechanism on flow, pressure, etc., in the isolated vascular system. In addition, this technique would be useful in separating vascular from cardiac effects of various drugs and hormones which influence the cardiovascular apparatus. This report describes a pump, which, while being substituted for either chamber of the heart, can be regulated over a wide range in respect to rate, output, and ejectile velocity.

Preliminary studies on dogs are being made with the diaphragm pump shown in Fig. 1. The pump is driven by a variable speed motor contained in an