tissue stroma with some slight infiltration of lymphocytes, plasma cells and large mononuclear cells. The cotton thread with which the kidney had been constricted was demarcated by a zone of connective tissue with occasional giant cells of the foreign-body type about the constricting thread which was usually imbedded in the kidney substance. A fairly diffuse and almost uniform involvement was observed in all of the kidneys to which a ligature had been applied. Occasional single damaged nephrons were also observed in the kidneys not ligated. The structural changes in the two kidneys were always different. Without exception, the involvement was more marked and more extensive in the kidney to which a ligature had been applied than in the opposite kidney.

*Discussion.* The animals for these studies were selected from those manifesting a maximal degree of hypertension and were killed while still in good physical condition. In all instances, the hypertensive state was of sufficient duration to produce a marked cardiac hypertrophy particularly of the left ventricle. Renal lesions apparently responsible for the hypertension were observed in every kidney to which a ligature was applied. Focal lesions of a slighter degree apparently due to the hypertension were observed in the opposite intact kidney. Though there were marked alterations in both heart and kidneys, no pertinent changes of any degree were observed in the large or small arteries of the heart, kidneys, spleen, liver, pancreas, lungs and brain. The inflammatory changes described by Cromartie[3] and these noted by Smith, Zeek, and McGuire[4] are explained by the fact that most of the animals used by Cromartie[3] died of pyogenic infection and suppurative lesions were common in those used by Smith *et al.*[4] The necrotic vascular lesions noted by Wilson and Byrom[1] were associated with an acutely developing severe hypertension and a rapidly fatal course. The findings of these authors are thus not applicable to chronic hypertension.

*Summary.* Varying degrees of chronic hypertension were produced in 16 rats by application of a figure-of-eight ligature to one or both kidneys. The animals were killed after the blood pressure had been maintained between levels of 150 to 240 mm of mercury for periods varying from 1 to 8 months. Microscopic examinations disclosed in each animal a marked cardiac hypertrophy particularly of the left ventricle. Renal lesions apparently responsible for the hypertension were observed in every kidney to which a ligature was applied and focal lesions of a slight degree apparently due to the hypertension were observed in the opposite intact kidney. No pertinent changes of any degree were seen in the large or small arteries of the organs examined.

## 15447

## Effect of Quick Freezing at Very Low Temperatures of Donor Tissue in Corneal Transplants.*

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There are 2 concepts regarding the necessity of using living instead of dead tissue in corneal transplantation. Castroviejo[1] and many others believe that living corneas must be used if successful, clear transplants are to be obtained. Others, Löhlein[2] and Salzer[3]

* The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University.

1 Castroviejo, R., *Am. J. Ophth.*, 1941, **24**, 1.

2 Lohlein, W., *Klin. Mon. f. Augenheilk.*, 1938, **101**, 106.

3 Salzer, R., *Archiv. f. Ophth.*, 1921, **105**, 469.

4 Weiss, P., and Taylor, A. C., PROC. SOC. EXP.

have the opinion that corneal grafts are invaded and replaced by the corneal cells of the host, the graft serves only as a framework or bridge, which temporarily guides and supports the corneal cells of the host and need not be living tissue. This group then regards the corneal graft as analogous to the nerve bridge graft in the manner in which it serves. An experiment using frozen corneas as donor tissue would be a test, though not conclusive, of the concept that corneal grafts need not be living.

Materials for nerve bridge grafts have been prepared by freezing, followed by dehydration, Weiss and Taylor.[4] These authors also transplanted frozen-dried rat corneas,[5] with promising results. Their grafts, observed for 6 weeks post-operatively, were clear but "resettled by host cells in supra-normal numbers."

Rapid freezing of donor eyes in liquid nitrogen also appeared to be a hopeful method of preservation of corneas for transplantation because it was thought that tissue frozen quickly at very low temperatures suffers a minimum of physical and chemical change.[6,7] If grafts of frozen tissue were unsuccessful it did not seem likely that drying, storage, and rehydration, as done with nerve grafts, would add to their chances of success.

Corneal transplants were made in rabbits using the technic described by Castroviejo.[1] The grafts were of the full thickness of the cornea and 5 mm square. The control series consisted of 22 cases in which the donor corneas were from normal animals sacrificed one to 2 hours prior to the transplantation and were therefore grafts of living tissue. In the experimental series, corneas were used which had been frozen in iso-pentane chilled with liquid nitrogen. Iso-pentane was used because of the observations of Gersh[6] that bubbles of gaseous nitrogen formed about tissue immersed in liquid nitrogen, thus slowing down the freezing process. Eight eyes were frozen in chilled iso-pentane, and transferred to liquid nitrogen within 15 to 30 minutes; 6 others were frozen directly in liquid nitrogen. The shift from iso-pentane was made because some of this compound clung to the tissue until it was transplanted, and it was thought that it might be an irritant and delay healing. However, no difference was detected in the behavior of grafts from donor eyes frozen in the 2 media, so the 2 groups may be considered as one.

The donor eyes were obtained as in the control series and frozen by plunging them into the freezing medium. These eyes remained in the liquid nitrogen for one or 2 hours, except in 4 cases in which they were stored 3 to 4 days. In every case the eyes were removed from the liquid nitrogen to room temperature and the graft cut within a few minutes, as soon as the cornea thawed sufficiently. At the time of cutting, the aqueous humor and the rest of the eye remained frozen, which was necessary in order that the globe might retain its shape, and the cornea be firm enough to cut. Cutting the transplant at this stage of thawing was much easier than in the living eye. All of the cuts could be made with a knife which left desirably smooth, straight edges. The grafts were absolutely clear and normal in thickness; however, they rapidly became thick and edematous when their cut edges came in contact with physiological saline solution. The grafts fitted the prepared opening in the host corneas perfectly. The frozen corneas were as good or better than fresh corneal material in so far as operative procedure was concerned. The corneal epithelium, however, loosened readily on these grafts and undoubtedly was soon lost.

The eyes of both the control and experimental animals were closed with a simple lid suture which was cut on the fourth day. In the control group, one animal died within 6 days following the operation. In 9 others the transplant either became dislodged or failed to heal satisfactorily. Failure to heal well appeared to be due to poor fit of the graft which was noted at the time of the operation. Gross infections did not occur. Twelve transplants were clear (Fig. 1) and have remained so to this writing, 6 to 7½ months later.

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BIOL. AND MED., 1943, **52**, 326.

[5] Weiss, P., and Taylor, A. C., *Anat. Rec.*, 1944, **88**, 49 (Suppl.).

[6] Gersh, I., *Anat. Rec.*, 1932, **53**, 309.

[7] Hoerr, N. L., *Anat. Rec.*, 1936, **65**, 293.

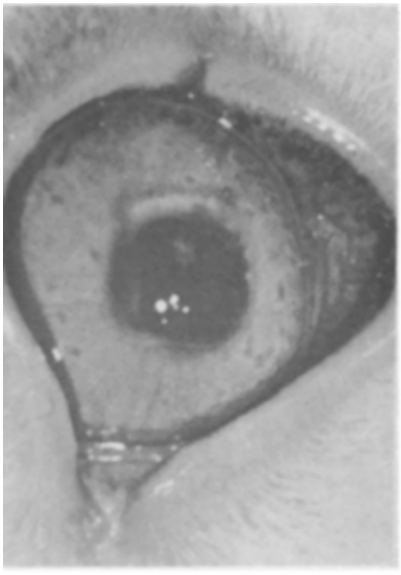

Fig. 1.
Transparent corneal graft 4 months after operation. The donor tissue was fresh, living cornea.

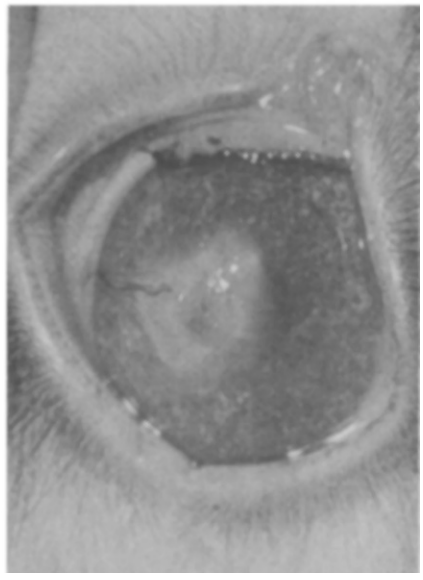

Fig. 2.
Corneal transplant 4 months after operation in which the donor tissue had been frozen in isopentane chilled with liquid nitrogen.

In the experimental group of frozen corneas the grafts were uniformly clear, healing well and as good generally as living grafts on the fourth day and therefore appeared hopeful. On the fifth, sixth, or seventh days the grafts become hazy, edematous, and the interface between graft and host became more distinct, deepening into a slight groove. The host tissue did not always become hazy, but blood vessels invaded the host cornea from the scleral border, a distance of about one millimeter. In several cases the center of the graft remained clear, while the peripheral portions became more and more hazy and edematous. The second week post-operative was a very difficult period in the healing of the graft, which appeared to behave almost as a foreign body. The grooves around the transplant were sometimes deep and it may be that in some a fistula formed, thus opening the anterior chamber for a second time. If this occurred it would account for the several synechias which formed in this group, though but once in the control series. All grafts of frozen tissue were opaque by the end of the second week and were becoming vascularized by the vessels which crossed from the sclera through the clear host cornea. The extent of this vascularization subsided somewhat by the fourth or fifth week, but all transplants were opaque and retained a slight blood supply. Six weeks to 2 months after the operation the eyes were not inflamed, the grafts were perfectly healed, the normal curvature of the cornea was restored, but the grafts were translucent or opaque (Fig. 2).

*Discussion.* The use of frozen corneas prepared in the manner described, for transplantation does not appear advisable. Their failure to persist as a transparent tissue suggests that efforts to preserve corneal tissue should be directed toward keeping the tissues alive for the necessary time. Freezing corneas in the manner described here appears to preserve the tissue in as nearly normal a condition as possible. The corneas were clear and not changed structurally but presumably they were no longer living. Certainly their structure as a bridge or framework would not be impaired by freezing. This procedure did not in any way make the technic of transplantation more difficult. At the time of operation and for 4 days thereafter these grafts appeared to be as good as grafts of living tissue.

It appears, therefore, that for the production of enduring, clear, corneal transplants,

viable tissues should be used. This implies that the stroma of clear, corneal transplants retains its identity as donor tissue, and is not replaced by the host within a short period. It may be that after some length of time some of the corneal cells die and are replaced by new cells derived either from the host or sister cells of the transplant. This process would only be that which may occur in a normal cornea to maintain the ordinary structure of the corneal stroma. Mitosis is exceedingly rare in the corneal stroma, but has been observed in colchicine-treated amphibian eyes (Peters[8]). This replacement may occur slowly, in rabbit eyes, as is suggested by the behavior of a few of the grafts in which the degree of opacity became reduced after 70 days, somewhat as the opacity of a heavy bond paper is modified by a drop of oil. The cornea did not become clear throughout its thickness and the improvement does not appear to be continuing at this time.

*Summary.* 1. Corneas frozen in liquid nitrogen can be grafted in rabbits, but invariably become opaque. Transplants of living corneal tissue, using the same technic as in the frozen, are successful and remain clear. 2. The use of frozen tissue is no handicap in the performance of the operation. 3. The results support the hypothesis that for the production of permanently clear corneal transplants living tissue must be used. 4. The data also support the suggestion that the corneal stroma of a clear graft retains its identity as donor tissue for a long period of time.

[8] Peters, F. J., *The Cytological Effect of Colchicine on the Cornea of* Triturus viridescens *and of Sulfanilamide on* Allium cepa, thesis, 1946, Fordham University.

## 15448 P

## Fibrinolytic Activity in Blood Serum During Pregnancy Complicated by Hypertensive Toxemias.

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The recent report by Smith and Smith[1] concerning fibrinolytic activity in the serum from pre-eclamptic and eclamptic patients and its absence in those with hypertensive disease with a superimposed pregnancy, suggests that there is a specific substance in the blood of the former groups which may be produced as a result of the toxemia. Should this prove to be true it would give a definite indication as to the direction toward which future research concerning the etiology of these conditions should be pointed and might well be utilized as a diagnostic test in certain obscure cases in which differentiation between the 2 conditions could not easily be made. In view of the fact, however, that the same activity was demonstrated in serum taken on the first day of menstruation and in post-operative patients, it seems more likely that this is a non-specific activity related to factors other than the pre-eclampsia.

In an effort to confirm this observation and to evaluate its application as a diagnostic procedure, bloods from a group of pregnant patients were tested for fibrinolysis.

*Material.* The patients were all attending the prenatal clinics of the Chicago Lying-in Hospital, the normal group being seen in the general clinic and the abnormal groups in the toxemia clinic. The normal patients demonstrated no signs which could be interpreted as indicating the presence of pre-eclampsia, with the exception of 2 who showed an excessive gain in weight. Those classified as

[1] Smith, O. W., and Smith, Geo. Van S., *Science*, 1945, **102**, 253.