that extracts of retinas induced vasoproliferation. 4’6

of desmosomes among the ectodermal cells during the
CAM development. The above results suggest that traction
chorioallantoic membranes (CAMs) of the developing chick
embryo were placed under tension with 10-0 nylon sutures
phase of the study, CAMs from 6- to 8-day-old chick em-
and subsequently examined histologically. In the initial
study, CAMs from embryos at each age from 7 to 16
days were placed under traction and sampled after 1 day. As
second study, CAMs from embryos at each age from 7 to 16
served as a mechanical stimulus for neovascularization in diabetic patients. 7’9 However, the effect
traction on the vasculature has not yet been invest-
gitated experimentally. In this report, in order to in-
vestigate the effect of traction on the induction or
acceleration of neovascularization, a tractional force
was exerted on the chorioallantoic membranes (CAMs) of developing chick embryos and the vascu-
lar changes were observed histologically. CAMs
have been used to study neovascularization, because
the vasculature is easily affected by numerous
stimuli.4’6-10

Materials and Methods. The CAMs of 6- to 17-
day-old fertilized white Leghorn chicken eggs, incu-
ated at 38°C, were used, in a manner that con-
formed to ARVO Resolution on the Use of Animals
in Research. The fertilized eggs were set upright in an
incubator at 38°C. On the 6th day after fertilization
or later, a window was made in the eggshell overlaying
the air sac and the CAM was exposed. The CAM was
placed under traction with 10-0 nylon suture in one
direction. The shell window was sealed with cello-
phane tape, and the egg was replaced in the incubator
at 38°C. The vascular changes of the CAMs were
examined histologically. As controls, some eggs had
windows but no other intervention, some eggs had a
window and 10-0 nylon suture placed without the
application of traction, and some eggs had a large

References
1. Anjou CIN: Influence of light on the 24-hour variation in
aqueous flare density and intra-ocular pressure in the normal
2. Krakau CET: On the connection between aqueous flow and
3. Rowland JM, Potter DE, and Reiter RJ: Circadian rhythm in
intraocular pressure: A rabbit model. Curr Eye Res 1:169,
1981.
4. Gregory DS, Aviado DG, and Sears ML: Cervical ganglionec-
tomy alters the circadian rhythm of intraocular pressure in
5. Rowland JM, Sawyer WK, Tittel J, and Ford CJ: Studies on
the circadian rhythm of IOP in rabbits: Correlation with
aqueous humor flow with intravitreal fluoresceinated dextrans.
7. Ericson LA: Twenty four hourly variations in the inflow of the
8. Reiss GR, Lee DA, Topper JE, and Brubaker RF: Aqueous
humor flow during sleep. Invest Ophthalmo Vis Sci 25:776,
1984.
9. Topper JE and Brubaker RF: Effects of timolol, epinephrine,
and acetazolamide on aqueous flow during sleep. Invest
10. Larson RS and Brubaker RF: Isoproterenol stimulates
aqueous flow in humans with Horner’s syndrome. Invest
11. Braslow RA and Gregory DS: Adrenergic decentralization
modifies the circadian rhythm of intraocular pressure. Invest
hole in the CAM. The resulting vascular changes in these controls were studied in the same fashion as the CAMs which underwent traction. All specimens were fixed in a phosphate-buffered 2.5% glutaraldehyde solution (pH 7.4), postfixed in 2% osmium tetroxide solution, dehydrated in a graded series of ethanol, and embedded in Epok 812® (Oken Shoji, Tokyo, Japan). The sections were observed by light and transmission electron microscopy (JEM 1200EX).

The sampling timing and sequence differed be-
Between the two experiments. In Experiment I, the age of the eggs and CAMs was held constant and the time under tension was varied. CAMs from 6- to 8-day-old eggs were placed under tension and sampled after half a day, 1 day and daily for a total of 7 days. In Experiment II, CAMs from eggs of various ages up to 16 days were exposed to the traction for the same length of time. CAMs from chicken eggs of 7 to 16 days old were placed under traction for 24 hr and sampled. Samples from both studies were fixed and observed histologically as previously described.

Results. Experiment I: The histological development of the CAMs in the control with the eggshells opened, but no other intervention is shown in Figure 1. The vascular network was noted in the CAMs macroscopically (Fig. 1a). In the 6-day-old CAM, capillaries were present in the ectoderm and few blood vessels were noted in the mesoderm by light microscopy (Fig. 1b). The electron micrograph of the ectoderm showed that there were only a few ectodermal cells around capillaries and junctions among them were scarce (Fig. 1c). Ectodermal cells and the desmosomes among them increased with the development of the chick embryos. By transmission electron microscopy, there were more cells around the capillaries and more desmosomes in the ectoderm of 12-day-old CAM in comparison with the 6-day-old CAM (Fig. 1c, d). Between the most superficial cells of the ectoderm of the 12-day-old CAM the junctional complex containing zonula occludens and zonula adherens (JC) is observed (×6000, bar = 1 µm). (c) Electron micrograph of ectoderm of Stage B' CAM. A few desmosomes (D) are noted among ectodermal cells. Many cytoplasmic organelles are noted in ectodermal cells (×8000, bar = 1 µm).

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The CAM placed under traction with nylon suture exhibited three types of histological pattern and density of blood vessels. In this report, they are called Stages A, B, and C, and are related to the time course.
In Stage A, there was no significant difference from controls. In Stage B, cell proliferation and migration from ectoderm to mesoderm was observed. Many cross-sections of small vessels were noted in the mesoderm along the ectoderm in this stage (Fig. 2a). By transmission electron microscopy, numerous cells were noted around capillaries. Between the most superficial cells of the ectoderm the junctional complex containing zonula occludens and zonula adherens was observed. Desmosomes among the ectodermal cells around the capillaries were not observed. Many cytoplasmic organelles were noted in the ectodermal cells; however, intermediate filaments were not observed (Fig. 2b). In some Stage B CAMs, only slight cell proliferation and migration were found. By transmission electron microscopy, a few desmosomes among the ectodermal cells were noted and the ectodermal cells contained many cytoplasmic organelles. Microvilli among the ectodermal cells were not noted (Fig. 2c). This subgroup of Stage B is called stage B' in this report. In stage C, numerous large vessels in parallel with the direction of the traction were noted on the portion of the CAM placed under traction compared with the opposite side of the CAM (Fig. 3a). Numerous vessels in the mesoderm were observed in the light micrograph (Fig. 3b). Numerous desmosomes among the ectodermal cells were noted in the transmission electron micrograph. Plenty of intermediate filaments in the ectodermal cells and microvilli among the ectodermal cells were noted; however, cytoplasmic organelles in the ectodermal cells were few (Fig. 3c).

Time course of these changes is summarized in the Table 1. This Table shows the number of eggs in each stage for each duration of traction. A Stage B response was observed from half a day to 3 days after the beginning of traction, and a Stage C response was observed after 3 days or later. A Stage B' response was observed 3 days and 4 days after the beginning of

Table 1. Stage of the response of the CAM blood vessels to traction which started on the 6th to 8th fertilized day

<table>
<thead>
<tr>
<th>Stage</th>
<th>Duration of traction (days)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>A</td>
<td>1</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
</tr>
<tr>
<td>B'</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
</tr>
</tbody>
</table>

Numbers of eggs exhibiting indicated stage in each duration are shown.
traction. The vasculature of the CAMs sutured with 10-0 nylon suture but not placed under traction was not different from that of the CAMs from eggs with eggshell windows and no further treatment. The vasculature of the CAMs with large holes, a control designed to investigate the reaction of the CAM to the wound, was not different from the reaction of the CAMs with the eggshells opened and no other treatments.

**Experiment II:** When the 7- to 9-day-old CAMs were placed under traction, a Stage B response was observed after 1 day following the initiation of traction. When 10- to 12-day-old CAMs were placed under traction, stage B' changes were observed after 1 day. When 13-day-old or older CAMs were placed under traction, no vascular changes were noted after 1 day.

**Discussion.** In the CAMs placed under traction with 10-0 nylon suture, ectodermal cells seemed to be activated, with an increase of cytoplasmic organelles and decrease of cytoplasmic intermediate filaments and intercellular junctions compared to controls in early stage, and later the density of mesodermal blood vessels was observed to increase. These results suggest that traction exerted on the CAMs induced the cell proliferation and migration observed in the Stage B response and the increase in the mesodermal vessel density observed in the Stage C response. As shown in Table 1, a Stage B' response was observed 3 to 4 days after the initiation of traction, at a time between that expected for a Stage B and Stage C response. This Stage B' may be a transient stage between Stage B and Stage C in Experiment I. These results cannot be immediately applied to the retinal vasculature in diabetic patients, but suggest that the traction on the vessels may provide a stimulus for vascular growth. The results of Experiment II suggest that the response of the CAM vasculature to traction differs according to the age of the CAM when the traction is applied. As the CAMs became older, the ectodermal cell response decreased. As shown in Figure 1, illustrating the control with only the eggshell window, desmosomes among the ectodermal cells around capillaries appeared to increase with the development of chick embryos. Junctional complexes containing zonula occludens and zonula adherens were observed among the most superficial cells of the ectoderm both with and without traction. Desmosomes are known to be sites of cell-to-cell adhesion, and this increase of desmosomes along the histological development may be related to the results of Experiment II. Tractional force alone cannot break the well developed intercellular junctions among the ectodermal cells. Retinal neovascularization is rarely observed even by histological examination in patients with proliferative vitreoretinopathy or macular pucker, which suggests that traction on the retina alone cannot induce neovascularization. This clinicopathological observation and the results of Experiment II suggest that traction on the vasculature is not an "angiogenesis factor," but a factor which may accelerate the neovascularization from the vulnerable vessels, in other words, a factor previously activated by other chemical factors.

**Key words:** chorioallantoic membranes, traction, changes of vasculature, histological examination

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**References**