only other compound which is known to lower the pain threshold is ergotamine tartrate. This agent lowers the tooth pain threshold and also the warm wire pain threshold.

Cortisone failed to raise the pain thresholds although euphoria was experienced by 2 of the 4 subjects. These results do not support the hypothesis that cortisone produces analgesia by the same mechanism as the opiates. Cortisone and the antipyretic analgesics have many similar actions. Similar metabolic and biochemical effects of cortisone and antipyretic analgesics are(7): 1. antipyretic effect, 2. glycosuria, 3 uricosuric effect, and 4. dilution of blood. On the glycosuria of the depancreatized rat, salicylates have an effect opposite to that of cortisone(8). Both cortisone and the antipyretic analgesics produce pain-relief only in certain selected clinical disorders. Sonnenschein and Ivy(9) found that the elevations of the tooth pain threshold after aspirin were not significant when compared with a lactose placebo. Studies in our laboratory confirmed this failure of aspirin to elevate the tooth threshold and further demonstrated the failure of aspirin to elevate the warm wire pain threshold. However, both the tooth and warm wire methods of algesimetry have demonstrated statistically significant pain threshold elevations in trained subjects with morphine and other analgesics. As cited above, cortisone and the salicylates are similar in many respects and this study indicates one further similarity in that neither produces an increase in pain thresholds.

The potent analgesics act on the physiologic system concerned with pain (the receptors, nerve fibers, synapses, or higher mental centers). However, pain-relief can be effected by agents that remove the pathophysiologic state causing the stimulation of pain receptors. These findings suggest that cortisone, like the salicylates, may produce pain relief by altering pathophysiologic states. That cortisone may alter pathophysiologic states is supported by numerous observations. With cortisone therapy there has been reported: a decrease in the joint swelling of rheumatoid arthritis and acute rheumatic fever; suppression of fever, skin eruption, and serositis in acute disseminated lupus erythematosus; subsidence of ocular inflammation; and a decreased edema in nephritis(10). Further speculation as to the mechanism of clinical pain relief by cortisone must await additional experimental investigation.

Summary. Under the conditions of these experiments, 11-desoxycortisone (25 mg) lowered the tooth pain threshold, but did not alter the wrist pain threshold, and cortisone (25 mg) did not alter the tooth or wrist pain thresholds. An alteration of the pathophysiologic state initiating pain is suggested as an explanation for the pain relief afforded by cortisone.

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Aqueous Humor as a Tissue Culture Nutrient.* (18917)

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(Introduced by Harry S. N. Greene.)
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Since Harrison(1) first performed experiments on the cultivation of cells *in vitro* using frog lymph, a variety of body fluids has been used in nutrient media. In view of the results of Greene(2) and Browning(3) on the transplantation of tumors and embryonic tissues to

the anterior chamber of rodents' eyes, a study was undertaken to evaluate aqueous humor as a tissue culture nutrient. The present report deals with results obtained with this medium in the cultivation of fibroblasts. The necessity of using a plasma clot has been avoided by the use of perforated cellophane in the case of fibroblasts, but the difficulty encountered in growing epithelial cells in the absence of a solid organic substrate has rendered results with these cells inconclusive as yet.

Materials and methods. The small size of rodents' eyes precluded their use as a source of aqueous humor. An inexpensive source of fairly large amounts of fluid was available in the eyes of adult cattle killed at a nearby slaughter house. Eyes were removed by attendants at the slaughter house without sterile precautions. In the laboratory the corneal surfaces of the eyes were bathed with 70% alcohol and the fluid was removed by means of a sterile 20 gauge hypodermic needle and syringe. Each eye yielded 1.5-2.0 cc of crystal clear fluid which did not clot on standing. Eyes in which either chamber had been damaged in removal or eyes which showed gross pathology were discarded. It was found convenient to store the fluid in the refrigerator without preservative in 10 or 20 ml aliquots. Bacterial contamination was rare when these simple technics were used. Several series of experiments in which aqueous humor was used were performed over a period of 1½ years, using both roller tubes and Carrel flasks. In the experiments to be reported, Carrel D 3.5 mm flasks were used. The cellophane was prepared after the manner of Evans and Earle(4) except that it was more convenient to insert the discs when wet and to sterilize the cellophane-containing flasks. The hearts from 12-day-old chicks were used as the explant material. These were cut into 4 pieces, each measuring about 2 mm square. One such fragment was placed in the approximate center of each flask, under the cellophane, after the addition of nutrient. The flasks were divided into 3 groups: (1) those containing aqueous humor only; (2) those containing embryo extract§ 20%, aqueous humor 80%; (3) those containing embryo extract§ 20%, horse serum‖ 40%, Earle's saline 40%. A total of 1.5 cc of nutrient was added to each flask just before the tissue fragment was put in place. The nutrients were changed twice a week by removing the fluid with suction, rinsing each flask with 1 cc of Earle's saline, and adding 1 cc of fresh nutrient. The cultures were incubated at 38.5°C without shaking or agitation. The flasks were not gassed, but phenol red indicator in the nutrient demonstrated that the pH remained close to 7.5.

Results. The fragments bathed in 100% aqueous humor showed migration of cells for the first 3 days. After this initial period further migration was not apparent nor was there unequivocal evidence of actual cell proliferation. The cells were clear in outline and contained very few droplets or unusual granulations. The cultures thereafter remained essentially static except for slow distintegration of the marginal cells. Despite the lack of activity, the cells of the explants survived at least 6 weeks as indicated by fixed preparations (Fig. 1).

In the group containing 20% embryo extract and 80% aqueous humor, cell migration and proliferation occurred early and persisted. The cell outline and clarity of cytoplasm re-

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§ Difco TC desiccated embryo extract; reconstituted with double distilled water and diluted 1:5 with Earle's saline. A cloudy suspension was formed which after centrifugation at 1500 RPM yielded a slightly cloudy supernatant.

‖ Difco TC desiccated horse serum; reconstituted with double distilled water. This resulted in a clear solution.

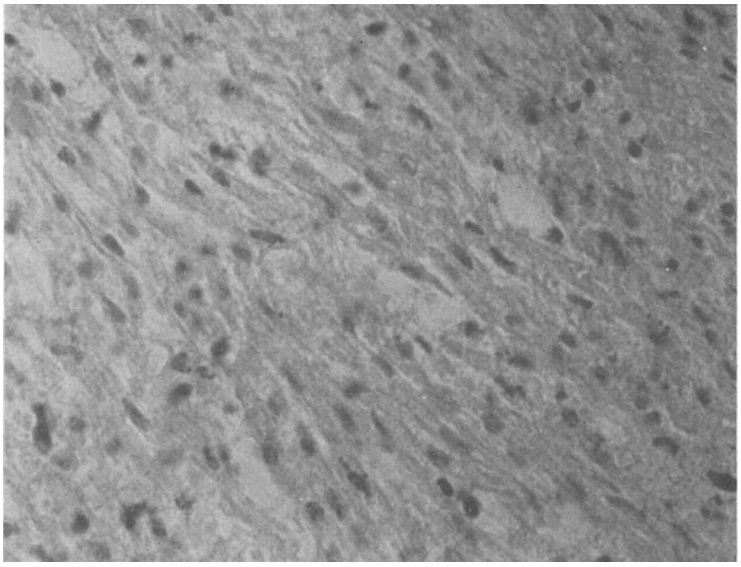

FIG. 1. Paraffin section through explant maintained in 100% aqueous humor for 6 weeks. Beginning necrosis is indicated by occasional pyknotic nuclei. H and E. × 300.

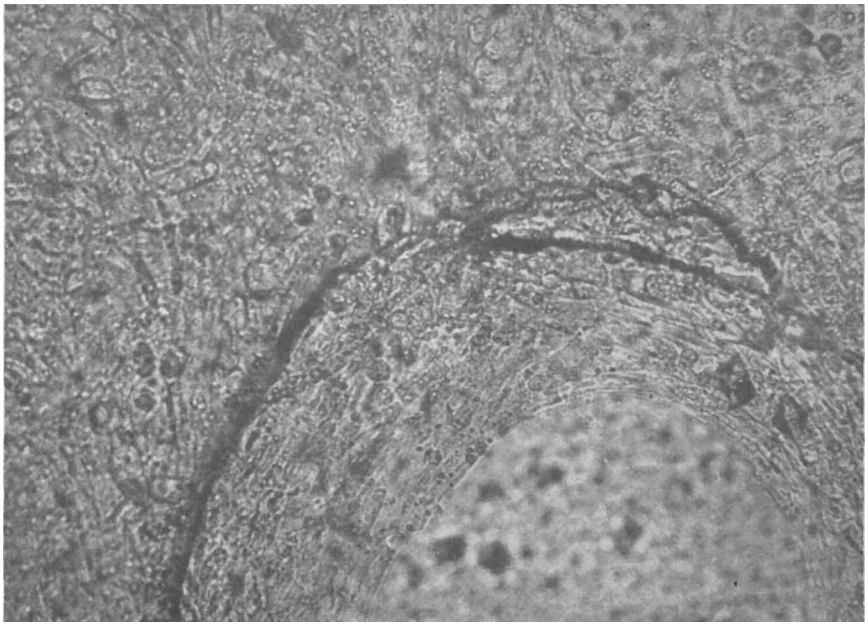

FIG. 2. Living culture under perforated cellophane in 80% aqueous humor and 20% embryo extract, photographed at 6 weeks. Cells are forming a characteristic whorl inside the perforation. Growth outside the perforation is several cells in thickness. × 175.

sembled that of cells grown in 100% aqueous humor. Growth occurred on both the glass and cellophane surfaces and tended to be in the form of continuous sheets, several cells in thickness (Fig. 2). Transplantation was performed at six weeks although the cells covered only about one-half of the flask area. Just prior to transplantation, the marginal cells had become somewhat granulated and proliferation appeared to be reduced. After transplantation, cells again migrated and proliferated from the transplanted fragment (Fig. 3).

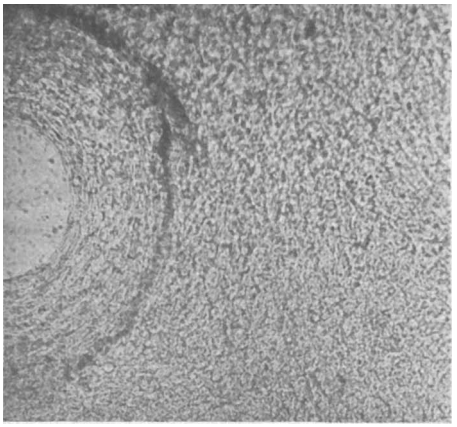

FIG. 3. Living culture under perforated cellophane in 80% aqueous humor and 20% embryo extract, photographed at 14 weeks, 8 weeks after transplantation. Luxuriant and uniform growth is evident. × 80.

In flasks which were continued without transplantation, cells were viable and active at 14 weeks although a zone of necrosis had appeared at the margins and was slowly increasing in width. Further observations over a longer period were not possible because of fungus contamination of the entire series.

Growth of fibroblasts from fragments placed in 20% desiccated embryo extract, 40% desiccated horse serum and 40% Earle's saline varied considerably from flask to flask, in contrast to the very consistent results obtained in both aqueous humor groups. In general, the cells in the former medium migrated slowly, tended to separate from the explant margin and to proliferate in the form of clumps of cells which later coalesced. The average area of growth produced at the time of transplantation was approximately one-half that produced in the group grown in aqueous humor and embryo extract, although growth in the best flask surpassed that of the aqueous humor group. On transplantation from this flask the superiority did not persist although migration and proliferation from the new transplants occurred earlier than from the aqueous humor transplants. The individual cells contained larger and more numerous vacuoles and granules and there was more variation in cell type than with the aqueous humor group. These observations have been confirmed in similar studies using roller tubes.

Discussion. The composition of aqueous humor has been the subject of considerable investigation(5). The ionic differences between it and serum are rather small and within the limits tolerated by living cells. The most striking differences are low protein (and low antibody) content and high ascorbate values found in aqueous humor.

The concentration of protein approaches the order of magnitude found in interstitial fluid. The importance of this factor is uncertain, but the correspondingly low antibody level may reduce inhibitory influences. The much higher levels of protein in serum may be the explanation for the toxic effects of some sera. The concentration of ascorbate in aqueous humor is about 12 times that in plasma. The specific vit. C levels required in fibroblast cultures are unknown, but large amounts of ascorbate are found in both embryonic and tumor tissue(6). This may possibly reflect higher needs of vit. C for optimal growth than are provided for in desiccated horse serum. In this laboratory the use of aqueous humor and desiccated embryo extract gave results superior to the desiccated horse serum embryo extract and saline mixture in respect to rapidity, consistency and extent of growth, uniformity of cell morphology and clarity of cytoplasm. It should be indicated that the results obtained using serum, saline and embryo extract reported in this series fall short of the extensive growth reported by Evans and Earle(4) using freshly prepared media. Possible explanations for this discrepancy may be the use of 12-day instead of 10-day embryos and 2 instead of 3 changes of fluid per week. Both of these factors were, however, applicable to all groups of the series. A further factor to be considered is the use of desiccated horse serum. Difco TC desiccated serum was used in the belief that since it had been tested and found satisfactory by the Tissue Culture Commission, it would furnish the most dependable control medium for comparison with aqueous humor. Horse serum

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is, however, known to vary considerably and, moreover, the drying procedure may possibly result in an inferior serum from the standpoint of volume of growth. On the other hand, Hetherington has reported excellent results with desiccated media(7,8).

The efficacy of aqueous humor as a tissue culture nutrient suggests that the success of intra-ocular transplantation depends on the ability of the fluid to maintain cells for relatively long periods or until vascularization occurs permitting continued growth.

Summary. (1) Pure aqueous humor from beef eyes will support survival but only limited growth of chick fibroblasts. (2) A mixture of aqueous humor and 20% desiccated embryo extract results in extensive proliferation of chick fibroblasts which is superior to that obtained in media prepared from desiccated horse serum, embryo extract and saline. (3) The possible explanations and significance of these findings have been discussed.

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Antithyrotoxic Effects of Antibiotics in Rats.* (18918)

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Several antibiotics have recently been shown to exert pronounced growth effects when fed in small quantities to chicks(1,2), pigs(3,4), or rats(5). These effects can be obtained on diets either adequate or deficient in vit. B_{12}(2,6), indicating that several factors may be made available through the action of the antibiotics. Ershoff(7,8) recently reported that supplements of dried penicillin mycelia or aureomycin mash, but not crystalline aureomycin HCl, prolonged the average survival rate of immature rats fed massive doses of thyroid powder. Since vit. B_{12} has been shown to be effective in counteracting the growth-retardation induced in young rats by administering large amounts of thyroid-active substances(9-11), it was of interest to see whether crystalline penicillin, neomycin or streptomycin would be similarly effective on rations either adequate or deficient in vit. B_{12}.

Procedure. In the first experiment, 50 young female albino rats (Carworth) were fed the following basal ration for 36 days: yellow corn meal, 35%; ground wheat, 25%; whole milk powder (Borden), 20%; linseed oil meal, 10%; alfalfa leaf meal, 6%; brewers yeast, 3%; and table salt, 1%. This ration is believed to contain an adequate supply of vit. B_{12},† and only one shipment of whole milk powder during the past 2 years was found to

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