demonstrated usefulness of repetitive subculture for detection of minimal quantities of virus has obvious practical implications. The advantages of each system may be combined for the isolation of rubella virus from clinical specimens. Both interference and CPE were demonstrable in PHA cells within 3 to 4 weeks after transfer of fluids from rubella infected AGMK cultures. We have encountered no agent other than rubella virus which produces interference in both systems and the unique rubella CPE in PHA cells.

It is generally accepted that 2 subcultures are sufficient for isolation of rubella virus when a AGMK culture system is employed. However, in one instance in this study, and rarely on other occasions, we have not demonstrated virus until a third blind passage had been performed in AGMK cultures.

Summary. AGMK and PHA tissue culture techniques are comparable systems for isolation of rubella virus from clinical specimens, although the AGMK system offers practical advantages. In order to demonstrate minimal amounts of rubella virus in either system multiple subcultures must be utilized. Employing Sindbis virus-interference, the agent of rubella may also be demonstrated in tissue explants in PHA cultures.

1. Parkman, P. D., Buescher, E. L., Artenstein, M. S., Proc. Soc. Exp. Biol. and Med., 1962, v111, 225.

2. Weller, T. H., Neva, F. A., ibid., 1962, v111, 215.

3. Alford, C. A., Neva, F. A., Weller, T. H., New Eng. J. Med., 1964, v271, 1275.

Received July 26, 1965. P.S.E.B.M., 1965, v120.

Influence of Miotics, Diamox and Vision Occluders on Light-Induced Buphthalmos in Domestic Fowl.* (30593)

JEAN K. LAUBER, JAMES MCGINNIS AND JAMES BOYD
Department of Animal Sciences, Washington State University, Pullman

Enlargement of the avian eye by continuous light has been reported(1). Several of our recent papers deal with attempts to characterize and explore further the nature of this light-induced buphthalmos(2).

This eye condition in domestic fowl resembles human glaucoma in many ways, yet it can be brought about by a relatively simple adjustment of the photoperiod. It was of interest to investigate the response of affected birds to several of the drugs used for glaucoma therapy. In addition, several types of vision occluders were used unilaterally, in order to test whether continuous light affects the eye by local or systemic route.

In Experiment 1, male broiler type chicks (Delaware × New Hampshire cross) were assigned on the day of hatching, 5 birds per group, to one of several treatments: 1. Control. Under a diurnal photoperiod of 14 hours light per day (14L/10D). 2. Experimental. Under continuous light (24L/0D), in the following groups: (a) Untreated. (b) Right eyelids sutured shut with nylon thread. The nictitating membrane was not sutured. (c) An "eye patch" affixed over the right eye to exclude light without applying pressure to the eye. This patch was made from a triangle, approximately 2 cm on a side, cut from a black-painted ping pong ball. The patch was lined with a thin layer of foam polyurethane and affixed to the skin by sutures at the 3 corners (Fig. 1). (d) Diamox acetazolamide (Lederle) given orally, 50 mg/100 g feed (*ca* 7 mg/chick/day). (e) Diamox, 500 mg/100 g feed (*ca* 70 mg/chick/day). (f) Floropryl (DFP), (Merck, Sharp & Dohme), 0.1% isoflurophate in anhydrous peanut oil, several drops in the right eye daily for 6 weeks. (g) Humersol (Demecarium bromide, Merck, Sharp & Dohme), several drops in the right eye daily. The stock solution, 0.25% aqueous, proved to be fatal

* Scientific Paper No. 2705. College of Agriculture, Pullman. Project 1677. This research was supported in part by grant NB-03770 from U.S.P.H.S.

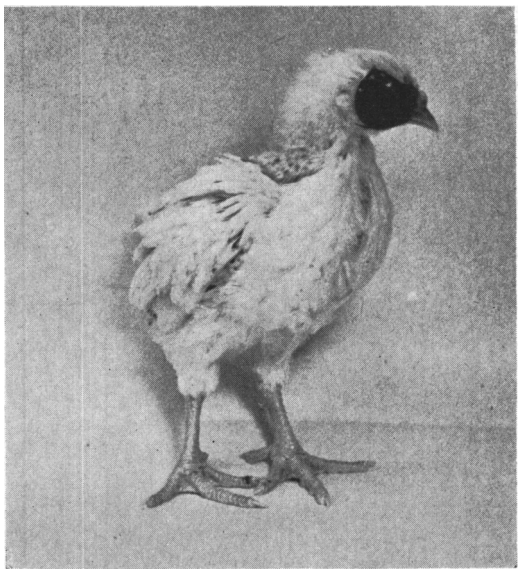

FIG. 1. Vision occluder made from a triangle of black-painted ping pong ball.

to several birds, and was diluted to 0.083% with saline for the final tests. (h) Phospholine iodide (Echothiopate Iodide, Campbell Pharmaceuticals), several drops in the right eye daily. The stock solution, 0.25% in mannitol, was toxic, so was diluted to 0.025% with saline.

These birds were maintained on their respective treatments from hatching to 6 weeks of age. They were kept in battery brooding cages equipped with electric heaters, and with food and water available at all times. All birds were weighed at weekly intervals. At sacrifice (by decapitation), either both eyes, or the right eye only, were enucleated, weighed, and fixed in Bouin's solution for later histological study.

In Experiment 2, "contact lenses" made for chickens were affixed to the eyes of male and female birds at 12 weeks of age. Half of the birds had been maintained under 24L/0D from hatching, and half under a diurnal schedule (14L/10D). These birds were reared in floor pens on a litter of wood chips. Heat was supplied during the early weeks by electric brooders. Appropriate food and water were available *ad libitum*. The eye covers used (kindly supplied by Mr. A. W. Schriner, Vision Control Research Co., Santa Rosa, Calif.), were made from translucent red plastic, and intended for use in cannibalism control, a serious problem in the poultry industry. They have an outer flange of reduced convexity, and are fitted under the eyelids, which hold them in place, but on top of the nictitating membrane. Eye covers were fitted on both eyes of 10 females and 2 males in each lighting treatment. The covers were not difficult to apply, but the birds seemed disturbed by them, and made repeated, often successful, attempts to scratch them out. After 4 weeks, only 5 birds retained one eye cover each. At the end of the experiment, at 6 months of age (3 months after contact lenses had been applied), one bird from each lighting treatment retained one eye cover each. These contact lenses were removed immediately prior to sacrifice. In the eye that had been covered, the iris was somewhat more vascular and the cornea slightly cloudy. The pupillary reflex remained patent, and both birds were markedly photophobic on the left (formerly covered) side. No gross inflammation was noted in the eyes that had been covered.

Results and discussion. Body weight. The accelerated growth usually seen in birds reared under continuous light failed to appear in the 24L/0D untreated birds in Experiment 1 (Table I). We have no explanation for this, except that the samples were small. In another and much larger experiment ($n = 100$), body weights at 6 weeks were 785 g for 24 hr, 740 g for diurnal. Eye weights ($n = 5$) were 2.27 g under continuous light, 1.83 g for 14L/10D birds.

The retarded growth of birds whose right eyelids were sutured shut, and of those fitted unilaterally with (ping pong) vision occluders, is probably related to their difficulty in finding the food and water supply. The ping pong treatment was the only one which caused mortality (after the tolerable doses of several miotics had been decided upon). The poor weight gain of the Diamox treated birds was presumably related to negative water balance.

Eye effects. Unilateral occlusion of vision resulted in even greater eye enlargement than on the contralateral (uncovered) side, or in control animals (Table I). It seemed possible that suturing of the eyelids, or use of "con-

TABLE I

Photoperiod	Treatment	Body wt (g)	Eye wt (g)	
			Treated eye	Contralateral eye
	6-wk-old males			
14L/10D	Control	845 (740)*	1.85 (1.83)*	—
24L/ 0D	Untreated	725 (785)*	2.06 (2.27)*	—
"	R. eyelids sutured	671	2.48	1.93
"	R. "ping pong" eye cover	672	2.29	1.83
"	Diamox, oral, low dose	643	1.65	—
"	" " high dose	568	1.50	—
"	Floropryl (R. eye)	744	2.04	1.88
"	Humersol (R. eye)	761	1.91	1.84
"	Phosphaline iodide (R. eye)	791	1.93	1.92
	6-mo-old females			
24L/ 0D	"Contact lens" (L. eye)		3.70	3.50
14L/10D	*Idem*		3.47	3.24

* More typical results from a separate and much larger experiment.

tact lens," might somehow exert pressure on the eyeball and contribute to its enlargement. However, enlargement of the covered eye occurred even with eye covers (made from ping pong balls) which did not fit closely against the orbit, and presumably exerted no external pressure.

Two of the 3 miotics used also caused a slightly greater buphthalmos in the treated as compared with the contralateral eye. These cholinergic drugs constrict the pupil and facilitate drainage of aqueous. Pupillary dilation in response to reduced light intensity is known to impair aqueous drainage. Birds reared under continuous light do in fact develop reduced corneal convexity and a narrow iridocorneal angle, which increases the possibility of angle block. However, 24L/0D birds have a smaller average pupil size than controls(2). The finding that chronic treatment with a miotic enhanced, rather than reduced, the buphthalmos usually seen in birds reared under continuous light mitigates against angle block as the primary causative factor in this condition. However, the possibility of pupillary block after treatment with miotics remains, and this might explain the excessive buphthalmos in miotic-treated eyes.

The observation that covering one eye caused no less buphthalmos than continuous light alone suggests that the ocular effects of continuous light in birds are general rather than local. The fact that the eye enlargement was even more extreme than on the contralateral side may reflect an additive effect: dilation of the iris into an already narrow angle would be expected to further impair aqueous outflow.

Diamox, a carbonic anhydrase inhibitor, is used therapeutically against glaucoma. Although the mechanism of action of this drug on the eye is unclear, it is presumed to act on the ciliary body, where aqueous is formed, and thus reduce intraocular pressure by reducing aqueous inflow. However, in many animals, a decrease in aqueous secretion is thought to be compensated by an increase in aqueous outflow resistance.

Sears(3) reported that Diamox was ineffective in lowering intraocular pressure in the unanesthetized hen, when given in doses roughly comparable to those used for humans. However, Seaman(4) later found that the intravenous dosage of Diamox required to reduce intraocular pressure in domestic fowl is approximately 100 times greater than for humans. In the present study, Diamox was given orally by mixing in the feed. Two dosage levels were chosen which appeared to bracket the levels found by others to be effective.

That both levels of Diamox used did have a systemic effect is evident from the reduced body weight, which was proportional to dosage. The eyes of Diamox treated birds were smaller than those of either 24L/0D untreated birds, or 14L/10D controls. Again, the eye effect was more pronounced with high

dosage of Diamox, though still evident with the low dosage. The response to Diamox suggests, although it does not prove, that the light-induced buphthalmos in domestic fowl is associated with excessive intraocular pressure.

Summary. Neither miotics nor vision occluders corrected the buphthalmos seen in chicks reared under continuous light, but Diamox caused a marked decrease in eye weight, proportional to dosage.

1. Lauber, J. K., Shutze, J. V., McGinnis, J., Proc. Soc. Exp. Biol. and Med., 1961, v106, 871.
2. Lauber, J. K., Shutze, J. V., Fed. Proc., 1963, v22, 576.
3. Sears, M. L., Arch. Ophthalmol., 1960, v63, 212.
4. Seaman, A. R., Himelfarb, T. M., A. J. Ophthalmol., 1963, v56, 278.

Received July 26, 1965. P.S.E.B.M., 1965, v120.

Studies on the Metabolism of Isoniazid in Subhuman Primates.* (30594)

J. H. PETERS,† G. R. GORDON AND P. BROWN
National Center for Primate Biology, University of California, Davis

Several studies have provided convincing evidence that the genetic polymorphism for isoniazid (INH) inactivation existing in human populations(1,2) is due to inherent capacities of human subjects to acetylate this drug to the metabolite, acetylisoniazid (AcINH)(3,4,5). Furthermore, a direct correlation between an individual's capacity to acetylate this drug, sulfamethazine (SMZ) (3,6) and hydralazine(3) was demonstrated. Unexpectedly, human subjects, whether exhibiting high or low abilities to form acetyl metabolites of the above drugs, were found to acetylate sulfanilamide(6) or p-aminosalicylic acid(7) to very nearly the same extent. Studies with liver tissue obtained by biopsy demonstrated a direct correlation between *in vitro* and *in vivo* capacities to acetylate the hydrazine derivatives and SMZ(3). However, the liver preparations were not active for converting sulfanilamide or p-aminobenzoic acid to acetyl metabolites(3). These studies have suggested that in human subjects different enzymes or tissues must be responsible for acetylating the group of compounds represented by the hydrazine derivatives and SMZ and the group comprising sulfanilamide, p-aminosalicylic, and p-aminobenzoic acids. Mechanistic studies to elucidate the bases for these observations would be greatly facilitated if an animal species could be found that exhibits a genetic polymorphism for acetylating substituted hydrazine derivatives such as INH or hydralazine, or aryl amines such as SMZ. Frymoyer and Jacox(8) reported a genetic polymorphism in rabbits for sulfadiazine acetylation. However, no clear polymorphism for acetylation was observed when INH was administered. Before their reports appeared, we had initiated studies with subhuman primates to find a species that exhibited varying capacities to acetylate INH like man.

Earlier studies, wherein plasma levels of INH and AcINH were measured in 6 rhesus monkeys shortly after intravenous or intramuscular administration of 5 or 20 mg INH/kg, suggested that these animals did not exhibit greatly divergent capacities to acetylate this drug(9). Later, 24-hour excretions of INH were determined following intramuscular injection of 10 mg INH/kg twice a day to 3 rhesus monkeys(10). These animals differed consistently in the amounts of unchanged drug eliminated in the urine. Thus, on 7 separate days of treatment, one monkey excreted between 10.4 and 12.9%, another between 8.0 and 9.5%, and the third between 5.4 and 7.0% of the daily dose. These results suggested that urinary excretion studies are more likely to demonstrate differences in inactivation capacities than determination of plasma levels of the drug.

This report describes investigations on the capabilities of rhesus and cynomolgus mon-

* This study was supported in part by Grants AI-05887 and FR-00169 from Nat. Inst. Health, U.S.P.H.S.

† Present address: Life Sciences Research, Stanford Research Inst., Menlo Park, Calif. 94025.