been adequately depicted. The RNA/DNA curve during lactation corresponded quite well with the limited RNA/DNA ratio data presented by Kirkham and Turner(13). The shape of the mammary gland RNA/DNA ratio curve reported in the present experiment is in almost perfect agreement with the shape of the mammary gland activity curves during lactation of glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase, 2 enzymes involved in the pentose phosphate pathway(14).

Summary. Total mammary DNA per 100 g of body weight increased 9.8% from day 20 of pregnancy to day 1 of lactation and 25.9% from day 1 to day 4 of lactation. Ovariectomy on day of parturition had no influence on mammary hyperplasia during early lactation. On the basis of total DNA per 100 g of body weight maximum mammary development occurred on day 8 of lactation (11.51 mg) and 24.8% of mammary growth occurred during lactation. No significant decrease occurred in mammary DNA until after day 24 of lactation. With the onset of lactation total mammary RNA increased much more rapidly than did total mammary DNA. Maximum mammary RNA content was observed on day 21 of lactation (75.06 mg) after which it declined precipitously to day 28 of lactation (39.61 mg). Mammary gland RNA/DNA ratio increased rapidly when lactation was initiated (1.24), attained its maximum on day 21 of lactation (2.95) and then decreased to day 28 of lactation (1.96). It is suggested that RNA/DNA ratios, when plotted against stage of lactation, represent the normal lactation curve of the rat.

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Scleral Contact Filters for Domestic Birds.* (28061)

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The effect of the electromagnetic spectrum (4000 to 7000 Å) on growth and development of avian gonadal tissue has been reviewed thoroughly by Withrow(1). However, the wave length-intensity relationships, as well as the efficiency of various wave lengths, require further investigation. More intensive work is also required on birds receiving no ocular illumination. The purpose of this research was to develop an inexpensive and relatively simple method for controlling wave length and light intensity for photoperiodic experiments with birds.

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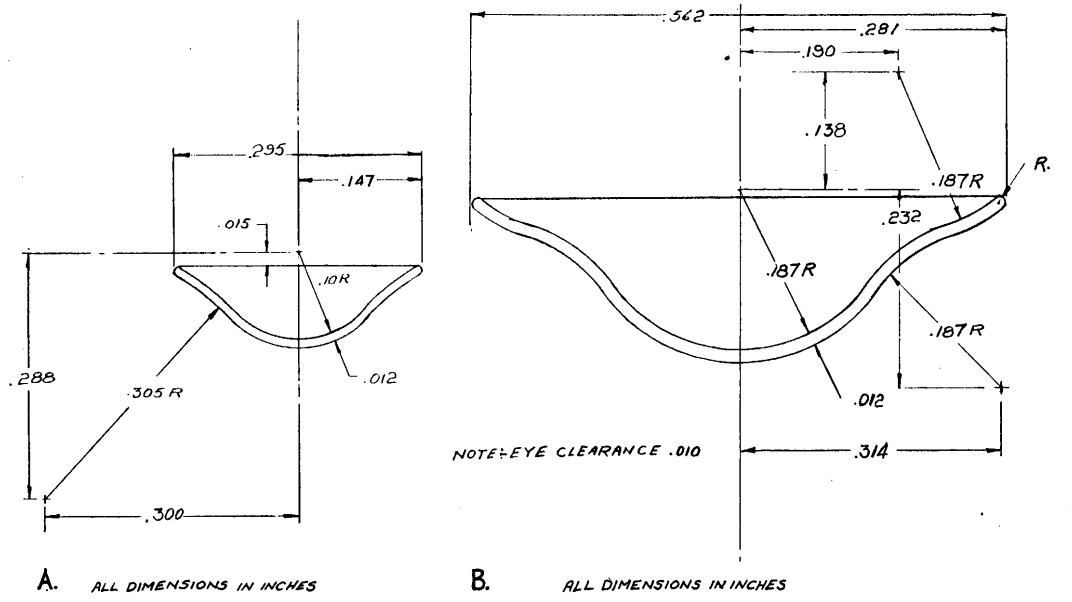

FIG. 1. Ocular characteristics for domestic bird. A. Japanese quail. B. Domestic fowl.

Methods and materials. Sexually mature SCWL laying hens *(Gallus domesticus)* and female Japanese quail *(Coturnix japonica)* were maintained under normal husbandry conditions (*i.e.*, 14 hours of light, adequate feed and water). To manufacture filters of a suitable shape, it was necessary to determine the size and shape of the eye, and for this purpose eyes were removed post-mortem from a number of birds, and measured. Prior to sacrificing the bird with an overdose of sodium pentobarbital, the eyes were examined for possible infection or other obvious pathological conditions and only eyes that appeared to be "normal" were used for the measurements.

Enucleation of the eye was completed within 10 minutes after pentobarbital injection. Incisions were made at both corners of the eye, to extend the palpebral fissure and expose the eyeball. Retraction of the eyelids with hemostats was followed by insertion of a cutting probe (Teat curet, Danish Model No. 806) to sever the optic nerve and the eye muscles (the internal, external, inferior and superior rectus muscles as well as the superior and inferior oblique muscles). The eyeball was then lifted out with a spoon-shaped spatula to avoid change in shape.

Paraffin molds of the eye were prepared immediately after removal of the eyeball from the orbit. The freshly enucleated eye was dipped several times (5 to 7) in a paraffin bath maintaind at 148°F. The resulting paraffin coating was cooled and then carefully raised off the eyeball with a pair of forceps.

The plaster of Paris imprints used for making the eyeball measurements were made by pouring fast-setting plaster of Paris into the paraffin shells. The hardened plaster of Paris was removed and placed in an optical comparator (Hilger & Watts Model No. TT695) for curvature measurements. The average curvature data from 15 different eyeballs (Fig. 1) were used to establish the form and shape of the brass template (Fig. 2). The eyes of the Japanese quail were found to be asymmetrical (Fig. 1A).

A brass template which was used to shape the contact filter was turned in a lathe to fit the specifications of Fig. 1. The surface was polished to a smooth finish to avoid roughness of the filter surface. A similar process has been described by Mischkin *et al.*(2).

The filters were made from sheets of cellulose acetate (0.0075 inch thick) by warming the brass template to approximately 300°F in corn oil and pressing it against the acetate (Fig. 3). The proper filter radius was obtained by using a cutting tool designed

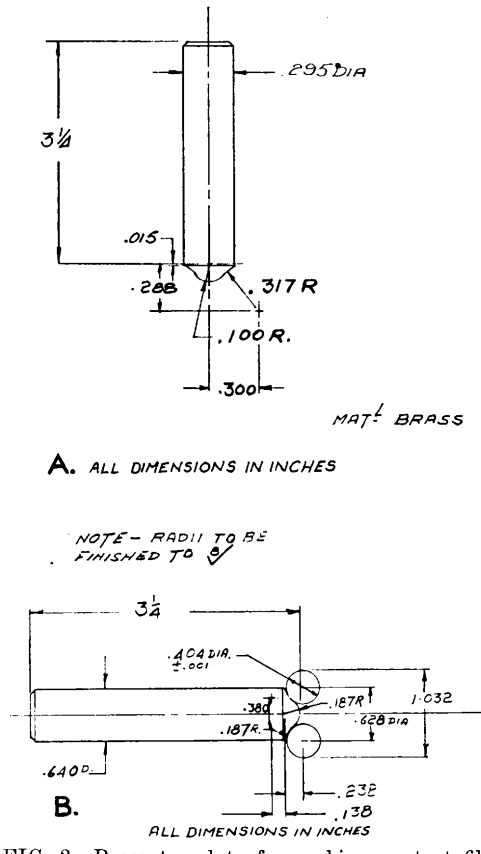

FIG. 2. Brass template for making contact filter. A. Specifications for Japanese quail. B. Specifications for domestic fowl.

for this purpose (Fig. 4). The final step, prior to cleaning and placing the lens in the eye, involved smoothing the outer edges with emery cloth (No. 240 Grit).

The contact filter was placed in position by first stretching the lower eyelid and placing the filter over the lower portion of the cornea and then stretching the upper eyelid and placing the filter over the upper portion of the eye. The final positioning of the filter can best be seen in Fig. 5.

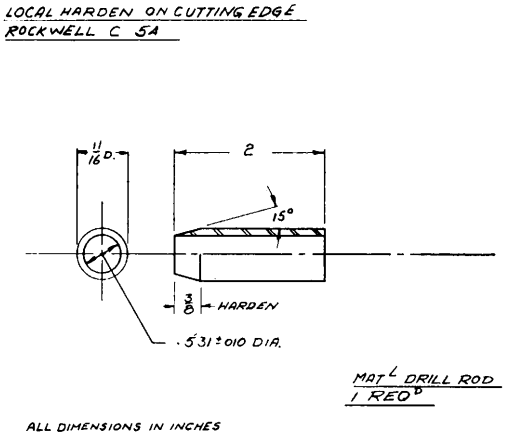

FIG. 4. Engineer's drawing of cutting tool for contact filters.

Results and discussions. Tests were made to determine whether removal of the nictitating membrane would be necessary. Before removal of the membrane, 2 drops of butacaine sulfate were placed in each eye and

FIG. 3. Shaping a piece of cellulose acetate with brass template.

FIG. 5. View of a SCWL hen's head with a red contact filter in position.

TABLE I. Adaptation Schedule for Group 1 (30 SCWL females). The pattern of Day 7 and 8 was repeated through Day 18 when the program was completed and both filters were left in place.

Day (exp period)	Wearing time (hr)
0	2
1	3
2	Rest
3	4
4	5
5	6
6	7
7	Left eye – 8; Right eye – 16
8	Left eye – 16; Right eye – 8

a 2-day post-operative period was allowed for recovery before the filters were inserted. There appeared to be a decrease in initial swelling when the eyes were bathed with an isotonic saline lubricant. However, for the birds observed (60 SCWL females) neither length of wearing time nor degree of irritation was improved by the absence of the nictitating membrane. If anything, presence of the membrane appeared to be beneficial as a source of natural lubricants. Therefore, in later experiments, the nictitating membrane was not removed.

Conditioning periods, with intermittent emplacement of the filters, were tried to see if the eye could adapt to the foreign material and prolong the total wearing time of the contact filters. Two different adaptation programs were undertaken, each with 30 birds (Tables I and II) in which the nictitating membranes had been removed. In Group 1, an 18-day adaptation program was undertaken (Table I). At all times the same filter was placed in the eye to minimize irritation due to slight differences between filters. Unless otherwise stated, filters were placed in both eyes at the same time. The nictitating membranes were removed 2 days before commencement of the adaptation program. The second conditioning period (Group 2) was somewhat less strenuous than that of Group 1 (Table II). The nictitating membranes had been removed from the eyes of these birds approximately 30 days prior to commencement of the adaptation program. Duration of the adaptation period in this group was much shorter than for the group described above. No difference in response was observed between the left eyes, wearing the filter 16 hours per day, and the right eyes, wearing the filters 8 hours per day. However, the birds' eyes had a better appearance overall (less swelling and less purulent exudate) than did the birds adapted on the Group 1 schedule. From these results, it was evident that repeated removal and insertion of the filters caused irritation to the eye, rather than an adaptation to the foreign material. In a third experiment the contact filters were simply placed in position with no previous adaptation. The eyes of these birds appeared normal even after a 6-month period in contact with the filter.

The radius and thickness of scleral filters appear to be the most critical factors in maintaining a "normal" eye for relatively long periods of time while wearing the contact filters. The filters used in the adaptation program of Group 1 were all of 0.0100-inch thickness except in 9 birds which had filters of only 0.0075-inch thickness. Although the overall adaptation program had resulted in considerable irritation to the eyes (puffiness and purulent exudate), the thinner filters caused much less irritation to the eye. In all subsequent experiments, the filters have been 0.0075 inch thick and have been found to cause little or no irritation to the eye. The filter diameter has also been found to be critical. A diameter of 0.531 inch seems to cover the eye sufficiently and at the same time fit without causing irritation around the edges of the filter.

Summary. It has been demonstrated that contact-scleral-filters can be placed in the

TABLE 2. Adaptation Schedule for Group 2 (30 SCWL females).

Day (exp period)	Wearing time (hr)
0	Left eye – 16
1	Left eye – 16 Right eye – 8
2	Left eye – 16 Right eye – 8
3	Left eye – 16 Right eye – 8
4 & 5	Rest
6	Left eye – 24 Right eye – 24

bird's eyes and remain for relatively long periods of time (up to 6 months), with no apparent damage to the eyes; by this means, the bird is exposed continuously to the spectral range transmitted by the filter. The wearing time decreases appreciably if the cornea is scratched during fitting or if the filters are too large in diameter or too thick.

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Effect of Purine Antimetabolites on Serum Globulins in the Rabbit. (28062)

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There is increasing evidence that the purine antimetabolites, 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG), are capable of suppressing antibody formation(1,2,3). We found that patients with nephrosis developed hypogammaglobulinemia during administration of 6-TG(4). These findings led us to investigate the effect of the purine antimetabolites on the serum proteins of experimental animals. This communication reports the changes in serum proteins, notably the fall in gamma globulin, associated with administration of these compounds to rabbits.

Material and methods. 6-MP* was prepared daily immediately prior to injection by dissolving 150 mg per ml of 1N NaOH; this was diluted with buffered saline† to pH 10-11 and a concentration of 37.5 mg/ml. 6-TG* (50 mg per ml of 1N NaOH) was prepared similarly and made to final concentration of 6.25 mg/ml. Control animals were given 1N NaOH diluted with buffered saline to pH 10-11.

Adult New Zealand albino rabbits were randomly distributed into 3 groups and injected intravenously daily for 10 days with: 18 mg 6-MP/kg body weight (6 rabbits), 2 mg 6-TG/kg body weight (6 rabbits), and NaOH-saline solution (2 control rabbits). Each control rabbit received the volume of solution used in the drug-treated groups. Animals were weighed on day 1 and day 11, and bleedings were *via* marginal ear vein on days 1, 6, 11, and 17; sera were stored at minus 20°C.

The same experimental plan was followed in guinea pigs except that 6-MP was administered at 50 mg/kg body weight per day and 6-TG at 5 mg/kg per day; injections were made intramuscularly into the paravertebral muscles, and blood samples were obtained by retro-orbital bleeding.

Total serum protein concentration was determined by the biuret method(5) using a Beckman B spectrophotometer. Paper electrophoresis was done using a Durrum type cell, veronal buffer 0.05M, pH 8.6 and stained with bromphenol blue(6). The paper strips were analyzed in a Spinco model RB analytrol using a B-4 cam and quantified on the basis of per cent and grams of protein. All determinations for each animal were made in the same cell at the same time. No correction factors were used for serum albumin, since the pre-injection serum served as the standard of reference for each animal.

Serum from each bleeding was studied by agar electrophoresis(7). Immunoelectrophoresis(8), as modified for microscope slides(9), was performed on pre-treatment and post-treatment bleedings. Sheep anti-rabbit sera were used to develop immunoelectrophoresis patterns. Absorption of sheep

* Kindly supplied by Dr. G. Hitchings, Burroughs Wellcome Co.

† Buffered saline at pH 7.4, 0.85% NaCl solution prepared (for 1 liter) as follows: M/15 Na_2HPO_4 - 84.1 ml; M/15 KH_2PO_4 - 15.9 ml; 8.5 g NaCl; 900 ml demineralized water.