Diet-related macular anomalies in monkeys

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Fundus color photographs and retinal fluorescein angiograms were obtained from 48 nonhuman primates of three macaque species. Yellow pigmentation of the macula was present in monkeys fed a standard laboratory diet containing xanthophylls but was absent in animals maintained on semipurified or liquid formula diets with no xanthophyll content. Plasma levels of xanthophylls ranged from 0.5 to 2.4 μl/ml in monkeys receiving the standard diet but were undetectable in animals raised on semipurified or liquid formula diets. Fluorescein angiograms revealed foveal areas of hyperfluorescence in almost all monkeys; however, the degree of hyperfluorescence was significantly greater in monkeys maintained on the semipurified or liquid formula diets.

Key words: xanthophylls, carotenoids, protein, semipurified diets, hyperfluorescence, macular pigment, macular axanthochromia

The metabolic significance of the macular pigment is still largely unknown. In pioneering studies, Wald[1] estimated the absorption of light by macular pigment in humans in vivo and established that its absorption spectrum in vitro was similar to that of "leaf xanthophyll."* Because all mammalian carotenoids are of alimentary origin,[2] macular pigment is presumably derived from ingested plants. It is therefore probable that one could use a xanthophyll-free diet to deplete the macular pigment and then establish the significance of this pigment by analyzing the ensuing anatomical and functional changes. This report describes diet-related depletion of macular pigment in a nonhuman primate.

Materials and methods

Animals. Three macaque species were studied (Table I). The rhesus and Japanese macaques were born at the Oregon Regional Primate Research Center (ORPRC). Cynomolgus macaques were purchased from commercial sources, and their ages were estimated; six were caught in Indonesia within a month of the observation period, and a seventh had been at the ORPRC for 6 years. Except for the Japanese macaques, the animals were caged indoors. The quarters were illuminated with fluorescent light during the daytime, the temperature was kept at approximately 26° C, and water was available at all times. A total of 24 macaques (11 rhesus, six Japanese, and seven cynomolgus) were fed Purina Monkey Chow (Ralston Purina Co., St. Louis, Mo.). Chow contains several plant foods, including wheat, corn, alfalfa, and soybeans. An additional 24 rhesus macaques were fed

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*What was once called "xanthophyll" is referred to in modern usage as "lutein" (3,3′-dioxy-α-carotene).[2-4] Leaf pigments occur as a mixture of substances: those very soluble in hydrocarbon solvents, called "carotenes," and those much less soluble in these solvents but very soluble in ethanol, called "xanthophylls." These two classes are grouped under the general term of "carotenoids."

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Table I. Experimental groups

Species	*Group*	*N(♂/♀)*	*Age (yr)*	*Years on diet*	*Diet*	*Protein (% kcal)*
Macaca mulatta (rhesus macaque)	Rh 1	6 (4/2)	7	5.5	High-protein SPD[A]	13.9
	Rh 2	5 (3/2)	7	5.5	Low-protein SPD[A]	3.7
	Rh 3	5 (3/2)	7	6.5	Chow[B]	15.6[C]
	Rh 4	8 (5/3)	3	3	High-protein liquid formula[D]	9.2
	Rh 5	5 (4/1)	3	3	Low-protein liquid formula[E]	3.0
	Rh 6	6 (0/6)	3	2.5	Chow	15.6[C]
Macaca fascicularis (cynomolgus macaque)	Cy 1	7 (0/7)	4-10	0.1-6	Chow	15.6[C]
Macaca fuscata (Japanese macaque)	Ja 1	6 (3/3)	2-3	2-3	Chow[F]	15.6[C]

[A]SPD, semipurified diet. See Table II for composition; until 1.5 yr., groups Rh 1 and Rh 2 received liquid formula diets similar to those for groups Rh 4 and Rh 5, respectively.
[B]Purina Monkey Chow with occasional fresh fruit.
[C]Calculated from manufacturer's proximate analysis.
[D]SMA infant liquid formula (SMA S-26; Wyeth Laboratories, Philadelphia, Penn.).
[E]Modified SMA (Portman et al. 1977[17]).
[F]Occasionally foraged for weeds.

Table II. Semipurified diets for 7-year-old rhesus macaques

	Concentration (gm/100 gm)	
	High-protein diet	*Low-protein diet*
Sucrose	37.2	44.7
Corn starch	9.3	11.4
Vitamin-free casein	13.0	3.5
Alphacel	7.4	7.4
Corn oil	14.9	14.9
Hegsted IV salt mix [A,B]	2.2	2.2
OWP vitamin mix[B,C]	1.1	1.1
Vitamin D_3 solution (2000 IU/ml)	0.1 (ml)	0.1(ml)
Water	14.9	14.9
% of calories:		
Protein	13.9	3.7
Carbohydrate	50.4	60.6
Fat	35.7	35.7

[A]See ref. 18.
[B]Obtained from Nutritional Biochemical Corporation, Cleveland, Ohio.
[C]See ref. 19.

semipurified solid or liquid formula diets which contained no unrefined plant products; two of these diets provided adequate dietary protein, and two were protein-deficient (Tables I and II).

Photographs and angiograms. Fundus photographs were obtained with a Carl Zeiss fundus camera (Model 31-03-40). Each monkey had its pupils dilated with 1% tropicamide (Mydriacyl; Alcon Labs, Inc., Fort Worth, Texas) and was then anesthetized with thiamylal (Surital; Parke, Davis & Co., Detroit, Mich.), approximately 25 mg/kg of body weight. Stereoscopic color fundus photographs of the posterior poles of both eyes were taken with Kodachrome 25 color film, KM-135-36. Stereoscopic fluorescein angiography was then performed with 10% fluorescein (Fluorescite; Alcon), 0.08 ml/kg, injected intravenously and flushed with 2 ml of saline. One eye of each monkey (usually the right one) was arbitrarily chosen to be photographed during the transit phase. Follow-up photographs were taken of the posterior poles of both eyes immediately after the transit phase and 5 min later. Direct funduscopic examination was conducted in a selected number of rhesus macaques.

Grading of macular findings. The term *macula*

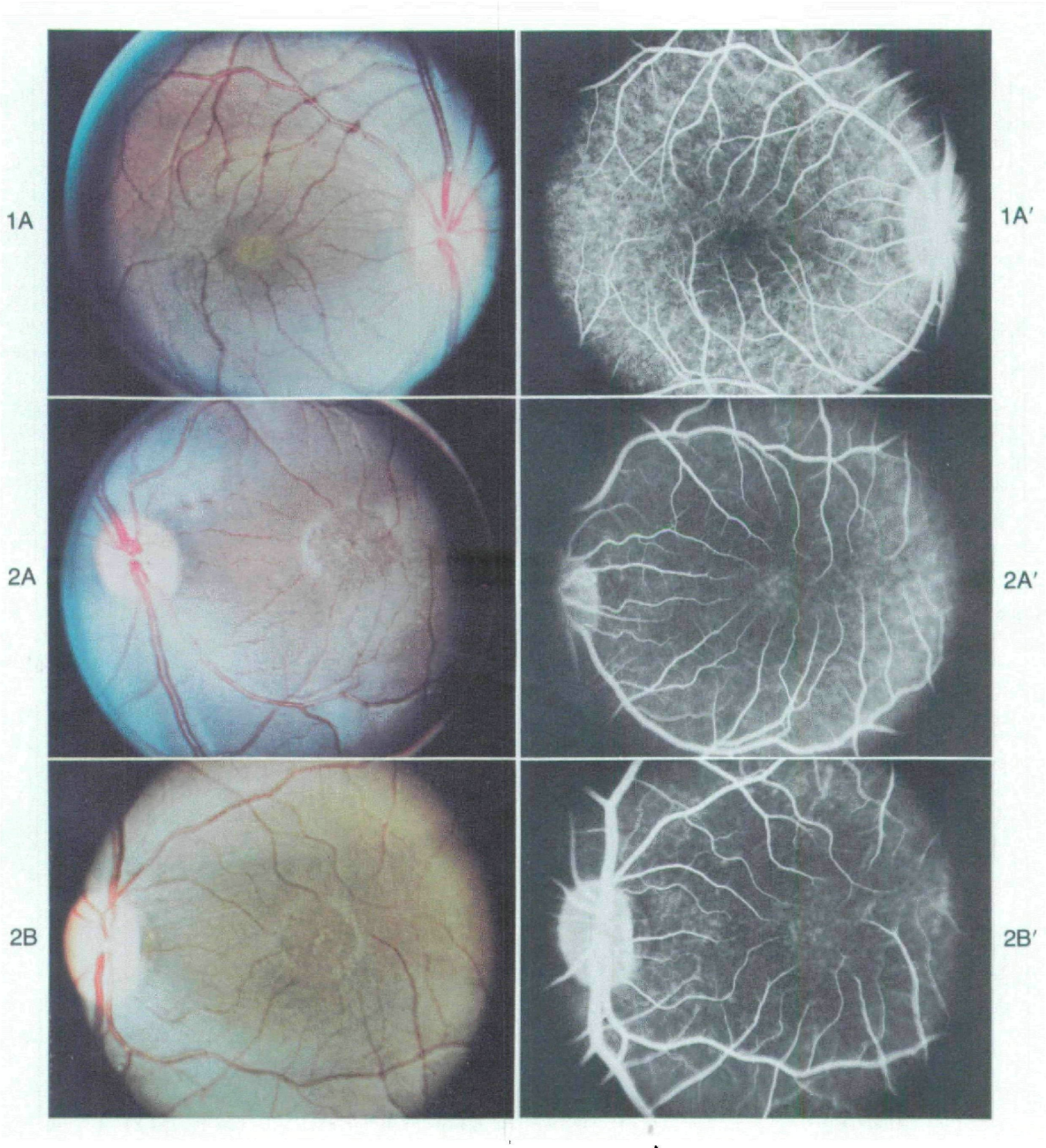

Fig. 1. Color photograph and retinal fluorescein angiogram of the posterior pole of the eye of a 7-year-old rhesus macaque (No. 5951) fed Chow. The photographs show a yellow macula (**A**) and minimal transmission of underlying choroidal fluorescence (**A**^{1}).

Fig. 2. Color photographs and retinal fluorescein angiograms of the posterior poles of the eyes of 7-year-old rhesus macaques fed semipurified diets (see Table II). The macular pigment is undetectable and drusen-like changes are seen (**A, B**); macular hyperfluorescence is extensive (**A**^{1}, **B**^{1}). The animal on the right (No. 5988; **A, A**^{1}) received the high-protein semipurified diet; the animal on the left (No. 5834; **B, B**^{1}) received the low-protein semipurified diet.

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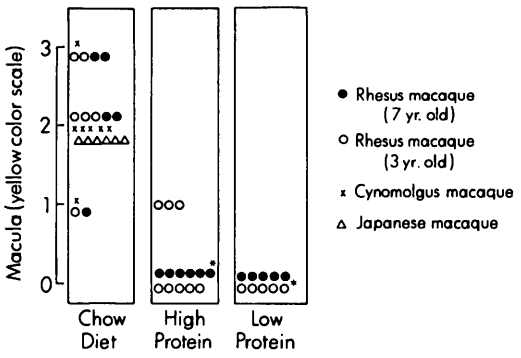

Fig. 3. Grading of macular yellow pigmentation: 0 = none; 1 = minimal; 2 = moderate; 3 = marked (see text). Each point represents one animal. In the two animals indicated with asterisks, a trace of yellow was detected by two of the four observers.

is used here to refer to an area approximately 1 optic disk in diameter, centered at the foveola. A representative color fundus photograph of each eye was graded independently by four observers on the basis of the amount of yellow in the macula as follows: 0 = none; 1 = minimal; 2 = moderate; and 3 = marked. When gradings were not unanimous, disagreements were recorded.

Positive transparencies were made of each angiogram. From these transparencies, a single frame, taken during the venous phase, was selected. The photographs were projected and graded as follows on the basis of the amount of hyperfluorescence in the macular area: 1 = none to trace; 2 = mild; 3 = moderate; and 4 = marked. The reliability of this method of grading was tested on the first 32 angiograms by three independent ophthalmologists. In the few cases where gradings were not unanimous, the three grades were averaged. The observers were unaware of the diets of the monkeys being graded.

Analytical procedures. Venous blood samples were obtained with dipotassium ethylenediamine tetraacetate (2 mg/ml) as an anticoagulant. Plasma was frozen and kept in the dark until analyzed, usually within 24 hr. A 3 ml volume of thawed plasma was saponified with 6 ml of 5% alcoholic KOH at 55° C for 30 min. Four successive extractions with approximately 7 ml of petroleum ether (30° to 60° C) were performed (5 min of shaking followed by 5 min of centrifugation). The solvent extracts were combined and evaporated under nitrogen. The residue was dissolved in 2 ml of heptane, and xanthophylls were partitioned into 2 ml of 82% ethanol. Light absorption of carotenes (in epiphase) and xanthophylls (in hypophase) was determined at 450 nm with a Beckman Model 25 spectrophotometer; concentrations were estimated by comparison with freshly prepared standards of zeaxanthin or β-carotene in 0.1M phosphate buffer (pH 7.4) and appropriate blanks. Readings with optical densities of 0.01 or less were considered below the limit of detection. Absolute values for carotenoids were established by the use of specific absorption coefficients,[5] corrected for absorption of zeaxanthin in 82% ethanol and of β-carotene in heptane and for the partition coefficients of carotenoids between the heptane and aqueous alcohol phases. Carotenoids in samples of food were assayed with a method similar to the one described for plasma, but 2 ml of water were added after saponification for better extraction.

Results

Ophthalmoscopy and color fundus photography (Figs. 1 and 2). All monkeys displayed optic nerve heads that were vertically oval, as is characteristic for macaques.[6] The disks were of normal color, and the cup-disk ratio was 0.3 or less in all animals. Myelinated nerve fibers were occasionally encountered in the peripapillary area. No specific vascular anomalies were present in any animal.

The macula (anatomic fovea) was defined in each animal by a distinct light reflex observable with ophthalmoscopy and fundus photography. It was approximately the same size as the optic nerve head and oval in shape, with its maximal diameter in the horizontal axis. At the center, a distinct foveolar light reflex was observed with ophthalmoscopy.

In the 24 Chow-fed monkeys, the central portion of the macula was yellow (Fig. 3). In stereoscopic fundus photographs, the yellow pigment appeared to be present at the level of the sensory retina. Mild mottling and occasional drusen-like bodies were present at the pigment epithelial level in some animals. There was no apparent influence of length of captivity, light regimen, species, age, or sex upon the appearance of the macula.

In the 24 monkeys fed semipurified or liquid formula diets, the most striking finding was a marked deficiency of the macular yellow pigment. It was totally absent in 19 animals, questionable in two animals, and pres-

Table III. Degree of foveal hyperfluorescence in retinal angiograms graded from 1 to 4

Species	*Foveal hyperfluorescence (mean ± S.E.)*		
	Chow diet	*High-protein diet*	*Low-protein diet*
Rhesus macaque	1.4 ± 0.2 (11)*	2.4 ± 0.3 (14)	2.7 ± 0.3 (10)
Cynomolgus macaque	1.7 ± 0.2 (7)	—	—
Japanese macaque	1.5 ± 0.2 (5)	—	—

p value (Student's t test): rhesus macaque col. 1 vs. 2, <0.02; 1 vs. 3, <0.01; 2 vs. 3, not significant.
*Number of animals in parentheses.

Table IV. Carotenoids in plasma and diets

Species	*Age (yr)*	*Plasma carotenoids (μg/ml, mean ± S.D.)*[A]					
		Chow diet		*High-protein diet*		*Low-protein diet*	
		Xanthophylls	*Carotene*	*Xanthophylls*	*Carotene*	*Xanthophylls*	*Carotene*
Macaca mulatta	3	2.40 ± 0.74 (14)	0.20 ± 0.05 (11)	0 (5)[B]	0.03 ± 0.02 (5)	0 (4)[C]	0 (5)[D]
	7	0.88 ± 0.66 (13)	0.14 ± 0.06 (13)	0 (5)	0 (5)	0 (5)[E]	0 (5)[E]
Macaca fascicularis	~4-10	0.47 ± 0.17 (6)	0.20 ± 0.12 (6)				
Diet content[F] (μg/gm)		21.72	0.71	0	0.05	0	0.10

[A] See Methods section. Comparison of Chow diet groups by Student's t test, row 1 vs. 2: xanthophylls, p <0.001, and carotene, p <0.02; row 2 vs. 3: not statistically significant. Number of animals in parentheses.
[B] Zero = below the limit of detection.
[C] One animal's values were unusually high and were excluded.
[D] Detectable (0.03 μg/ml) in one animal.
[E] Plasma samples were pooled.
[F] High- and low-protein diet values are for liquid formula diets only.

ent to a minimal degree in three animals (Fig. 3). Also, drusen-like bodies at the level of the pigment epithelium were quite prominent in the maculae of many of these animals, in contrast to their infrequent occurrence in the Chow-fed group.

Fluorescein angiography. Areas of macular hyperfluorescence were observed in almost all monkeys (Figs. 1 and 2). They varied from small traces localized in the fovea to intense fluorescence involving large portions of the macula. No leakage of dye was observed in any eye. The pattern of macular hyperfluorescence showed no consistent relationship to age, sex, species, illumination, or length of captivity. However, an effect of diet was again seen in the rhesus macaques. Because no age effect was detected, the observations on 3- and 7-year-old rhesus macaques were combined. Table III shows that the hyperfluorescent areas were more intense and extensive in the monkeys raised on semipurified and formula diets than in the Chow-fed animals. Animals fed low-protein rations showed a slight, but not significant, increase in hyperfluorescence over those fed high-protein rations. No abnormalities of the optic nerve were observed.

Determination of carotenoids. Recovery of carotenoids added to plasma or buffer solutions was quantitative. The partition of zeaxanthin in heptane:82% ethanol was 21:79; less than 1% of β-carotene was detected in the alcohol phase. The absorption spectrum of the aqueous alcohol and heptane plasma extracts showed the typical maxima characteristic of xanthophylls and carotenes, respectively.[5] The plasma concentrations of carotenoids determined in rhesus and cynomolgus macaques are shown in Table IV. Among Chow-fed animals, plasma levels of xanthophylls and β-carotene were significantly higher in 3-year-old than in 7-year-old rhesus macaques. Values for Chow-fed adult

cynomolgus macaques were lower than those for 7-year-old rhesus macaques, but the difference was not statistically significant. Plasma levels of xanthophylls were below the limit of detection in rhesus macaques of either age fed semipurified or liquid formula diets. Carotene was also undetectable in 7-year-old rhesus macaques fed semipurified diets and was measurable but low in 3-year-old animals receiving liquid formula diets.

The concentrations of carotenoids in food are also shown in Table IV. Xanthophylls were present at a concentration of 21.72 μg/gm in the Chow diet but were undetectable in the liquid formula diets. Carotenoid contents of the semipurified diets could not be determined because of the presence of substances that interfered with the absorption spectra of zeaxanthin and β-carotene; these diets most likely contained no carotenoids, since xanthophylls were not present in the constituents and vitamin A acetate, but not β-carotene, was added during preparation.

Discussion

Polyak[7] and Wolin and Massopust[6] reported the presence of a macula lutea in an extensive series of nonhuman primates. We have confirmed these findings in rhesus macaques and have documented the presence of a macula lutea in two additional species, Japanese and cynomolgus macaques. Although the degree of yellow macular pigmentation varied among monkeys fed Chow, we could not detect differences correlated with age, sex, species, light source (artificial or natural sunlight), or length of captivity (between 1 month and 7 years). In contrast to the yellow color of the macula in all Chow-fed monkeys, no yellow macular pigmentation was seen in most monkeys raised on semipurified or liquid formula diets. These diets contained no xanthophyll and resulted in undetectable levels of xanthophyll in plasma.

The early studies of Wald[1] demonstrated that the absorption spectrum of macular pigment was similar to that of xanthophyll or, more specifically, leaf lutein. M. L. J. Crawford (personal communication) has recently confirmed Wald's finding by identifying lutein microspectrophotometrically in the macula lutea of humans and monkeys. Our results, demonstrating dietary depletion of the macular pigment, strongly suggest that this pigment is xanthophyll of alimentary origin. We proposed the term *axanthochromic macula* to describe the absence of normal yellow macular pigmentation.

Several investigators[8–11] have used fluorescein retinal angiography in monkeys, but studies involving dietary modifications or comparisons between species have not been attempted. We have observed the occurrence of circumscribed areas of hyperfluorescence, resembling human macular "window defects,"[12] in the foveas of most of the monkeys we examined. We were unable to relate differences in intensity or extent of these areas to the type of illumination or length of captivity, factors that might provide an estimate of the influence of natural vs. laboratory conditions. Thus it seems likely that foveal hyperfluorescent areas are present in wild monkeys and are normal variants in these species. This suggestion is supported by the observations of El-Mofty et al.,[13] who reported window defects in four out of four free-ranging rhesus macaques on which angiography was performed; the areas of hyperfluorescence correlated with ophthalmoscopically visible mottling, which was detected in fully half of the 105 monkeys examined. Examination of the maculae of our Chow-fed cynomolgus macaques by electron microscopy suggested that some hyperfluorescent areas may correlate with reduced melanin content of pigment epithelial cells.[14] Other hyperfluorescent areas, associated with small, yellow-white drusen-like bodies seen clinically, may represent lipoidal degeneration of pigment epithelial cells, as demonstrated by Fine and Kwapien.[15]

Beyond the finding of widespread macular hyperfluorescence in three macaque species, we have seen a significant increase in the degree of hyperfluorescence in rhesus macaques fed semipurified and liquid formula diets. This increase probably arises from alterations at the pigment epithelial level, al-

though absence of the screening effect of macular pigment could be a contributing factor.

The functional significance of the macular anomalies seen in these macaques remains to be established. Animals with both macular axanthochromia and hyperfluorescence showed no gross visual disturbance, although the amplitude of the cone electroretinogram was reduced in monkeys with macular axanthochromia raised on low-protein semipurified diets.[16] The observations reported here suggest the usefulness of nonhuman primates for examining the significance of macular pigment and, more generally, for investigating the effects of diet on the structure and function of the macula.

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REFERENCES

1. Wald G: Human vision and the spectrum. Science **101**:653, 1945.
2. Goodwin TW: The Comparative Biochemistry of the Carotenoids. London, 1952, Chapman & Hall, p. 356.
3. Strain HH: Leaf Xanthophylls. Washington, D. C., 1938, Carnegie Institute.
4. Moss GP and Weedon BCL: Chemistry of the carotenoids. *In* Chemistry and Biochemistry of Plant Pigments, ed. 2, Goodwin TW, editor. London, 1976, Academic Press, Inc., vol. 1, p. 149.
5. Davies BH: Carotenoids. *In* Chemistry and Biochemistry of Plant Pigments, ed. 2, Goodwin TW, editor. London, 1976, Academic Press, Inc., vol. 2, pp. 38-165.
6. Wolin LR and Massopust LC Jr: Characteristics of the ocular fundus in primates. J Anat **101**:693, 1967.
7. Polyak S: The Vertebrate Visual System. Chicago, 1957, University of Chicago Press.
8. Hill D: Fluorescence angiography. Trans Ophthalmol Soc UK **88**:187, 1969.
9. Perry HD, Hatfield RV, and Tso MO: Fluorescein pattern of the choriocapillaris in the neonatal rhesus monkey. Am J Ophthalmol **84**:197, 1977.
10. Henkind PM, Bellhorn RW, Murphy ME, and Roa N: Development of macular vessels in monkey and cat. Br J Ophthalmol **59**:703, 1975.
11. Engerman RL: Development of the macular circulation. INVEST OPHTHALMOL **15**:835, 1976.
12. Gass JDM: Stereoscopic Atlas of Macular Diseases: Diagnosis and Treatment, ed. 2. St. Louis, 1977, The C. V. Mosby Co.
13. El-Mofty A, Gouras P, Eisner G, and Balazs EA: Macular degeneration in rhesus monkey *(Macaca mulatta)*. Exp Eye Res **27**:499, 1978.
14. Feeney-Burns L, Malinow MR, Peterson L, Klein M, and Neuringer M: Macular hyperfluorescence in monkeys. Presented at Western Section Regional Meeting, Association for Research in Vision and Ophthalmology, Seattle, October 1978.
15. Fine BS and Kwapien RP: Pigment epithelial windows and drusen: an animal model. INVEST OPHTHALMOL VIS SCI **17**:1059, 1978.
16. Neuringer M, Denney D, and Sturman J: Reduced plasma taurine concentration and cone electroretinogram amplitude in monkeys fed a protein-deficient semipurified diet. Am J Clin Nutr **32**:xxvi, 1979 (abst.).
17. Portman OW, Alexander M, Neuringer M, Novy M, Illingworth R, and Uno H: Effects of perinatal malnutrition on lipid composition of neural tissues from rhesus monkeys. J Nutr **107**:2228, 1977.
18. Hegsted DM, Mills RC, Elvehjem CA, and Hart EB: Choline in the nutrition of chicks. J Biol Chem **128**:459, 1941.
19. Portman OW, Alexander M, and Maruffo CA: Nutritional control of arterial lipid composition in squirrel monkeys: major ester classes and types of phospholipids. J Nutr **91**:35, 1967.