

close proximity to one or more additional receptors, that is, within 200 to 400 Å. On liver plasma membranes, however, most of the receptors occurred singly or in a few groups of three or more. To determine whether these few groups of receptor sites were a result of ligand-induced aggregation, we examined liver plasma membranes that were fixed with 1 percent glutaraldehyde, washed, and subsequently incubated with Fm-I. Table 1 shows that the distribution of Fm-I was identical on both the control and prefixed membranes. When the multivalent ligand ferritin-concanavalin A (F-Con A) was incubated with fresh liver plasma membranes, large aggregates of ferritin particles were observed. Fixation of the liver membranes with glutaraldehyde totally blocked ligand-induced aggregation by the F-Con A (data not shown). Similar findings with both Fm-I and F-Con A were reported for adipocyte plasma membranes (3). The ability of glutaraldehyde to prevent the ligand-induced aggregation of F-Con A receptor sites and the data showing no difference in Fm-I distribution on control or prefixed membranes indicate that Fm-I binding to the insulin receptor did not cause aggregation of insulin receptors in either tissue.

The morphological data in Fig. 1 constitute proof of a major difference in the ultrastructural organization of the insulin receptor on adipocyte and liver plasma membranes. The adipocyte insulin receptors occur primarily in natural groups prior to insulin binding. However, most of the insulin receptors on liver plasma membranes occur singly. Bergeron *et al.* (6), using electron microscope radioautography, reached a similar conclusion, stating that there was no preferential localization of receptors over the hepatocyte plasmalemma to which insulin had access, and that there was no obvious clustering of grains. The morphological difference in the distribution of insulin receptor sites on adipocyte and liver plasma membranes are consistent with the biochemical differences reported for [<sup>125</sup>I]insulin binding by Shechter *et al.* (1) and Schweitzer *et al.* (2).

Cross-linking or aggregation of the insulin receptor has been suggested as an important part of the initiation of insulin's action (7). This conclusion was based on data demonstrating the insulin-like properties of bivalent antibodies to the insulin receptor or other membrane components and the inability of monovalent forms of these antibodies to mimic the action of insulin. Morphological data indicate that Fm-I does not cause aggregation of insulin receptors at the ultra-

structural level in adipocytes, liver, and placenta, even though the Fm-I is biologically active (3). This suggests that if insulin, as a monovalent ligand, causes a cross-linking to occur that helps elicit a biological response it must be by a different mechanism than aggregation of receptors. We propose a different model for insulin's action at the receptor level. Perhaps the natural presence of groups of insulin receptor sites on the adipocyte increases the probability of a cross-linking reaction that may be necessary for or may intensify insulin action. This could help explain the greater insulin sensitivity of adipocytes compared to liver where the receptor sites are not grouped. Such grouping of receptor sites on adipocytes might allow the insulin occupancy of one site to activate adjacent sites. This molecular cross-linking might occur between the disulfide bonds of insulin and the sulfhydryl groups in the insulin receptor.

Schweitzer *et al.* (2) have shown that the adipocyte membrane has a greater number of accessible disulfide bonds than liver and that conversion of these to sulfhydryl groups increases insulin binding. Recent data (8) show that opiate receptor function can be modulated through an oxidation-reduction mechanism; thus the oxidation of thiol groups by cupric ions mimics the action of morphine and those ions even compete for binding. Since several oxidizing agents, such as H<sub>2</sub>O<sub>2</sub>, vitamin K, and diamide, mimic insulin activity (9), bivalent anti-

bodies may mimic the action of insulin through a similar oxidation-reduction reaction between the disulfide bonds of the antibody and sulfhydryl groups of the receptor, and not because of aggregation. The role of disulfide bonds and sulfhydryl groups in the initiation of insulin action must be substantiated by further experiments.

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10. Supported by NIH grants AM 20097 and T32 AM 07296.

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26 June 1980; revised 5 September 1980

## Gyrate Atrophy of the Choroid and Retina: Improved Visual Function Following Reduction of Plasma Ornithine by Diet

**Abstract.** In a patient with gyrate atrophy of the choroid and retina, an arginine-deficient diet has reduced plasma ornithine concentration fivefold during the past 20 months. Subjective improvement in her visual function was noted approximately 15 months after institution of the diet. This has been documented by improvements in the electroretinogram, dark-adaptation, and color vision. The improvement involves rod and, to a lesser extent, cone function. The results, although preliminary and limited to a single patient, suggest that reduction of plasma ornithine with a low arginine diet is beneficial in this disease.

It is generally accepted that no therapy has been successful in the treatment of degeneration of the choroid and retina. This group of diseases is characterized by inexorably progressive loss of visual function leading to eventual blindness. The purpose of this report is to document the sustained improvement in visual function in a patient with one form of chorioretinal degeneration, gyrate atrophy.

Gyrate atrophy (GA) of the choroid

and retina is an autosomal recessive chorioretinal degeneration that ultimately results in severe visual deficits expressed by high myopia, progressive concentric constriction of the visual field, cataracts, pathologically reduced dark-adaptation, extinguished electroretinogram (ERG), and abnormal electrooculogram (EOG) (1).

In 1973, Simell and Takki reported that GA patients have 10- to 15-fold increases in plasma ornithine concentra-

tions with resultant overflow ornithinuria (7). Subsequently, several investigators have shown a deficiency of ornithine- $\delta$ -aminotransferase (OAT; E.C. 2.6.1.13) activity to be the primary abnormality in GA (2-6). Since OAT normally degrades ornithine (of which arginine is a precursor), a deficiency of this enzyme coupled with an arginine intake in excess of that required for protein synthesis results in the massive accumulation of ornithine characteristic of GA. For the past 20 months, we have treated one GA patient, a 36-year-old female whose clinical features have been previously reported (6), with a low arginine diet. The metabolic effects of this regimen are documented elsewhere (7). We now report the ophthalmologic results of this therapy.

Some GA patients have had a partial reduction in plasma ornithine when treated with pharmacologic amounts of pyridoxine (8, 9). Like the majority of GA patients, however, our patient did not respond to high-dose pyridoxine therapy (7). In February 1978, we therefore began her on a low arginine diet consisting of approximately 10 g of natural protein supplemented with 20 g of a mixture of essential amino acids (7). On this diet, her plasma ornithine decreased fivefold over 6 weeks from concentrations of  $964 \pm 35 \mu\text{M}$  (mean  $\pm$  standard error of the mean,  $N = 6$ ) to concentrations ranging, for the subsequent 20 months, from  $355$  to  $24 \mu\text{M}$  with a mean of  $176 \pm 18$  ( $N = 28$ ).

Because the pathophysiology of the chorioretinal degeneration characteristic of GA is not understood, the effects of reducing plasma ornithine on the ophthalmological abnormalities could not be predicted. Therefore, before instituting the diet, we made detailed measurements of the visual function and fundal abnormalities of our patient; these measurements included ophthalmoscopy, fundus photography, visual acuity, dark-adaptometry, color vision, Ganzfeld ERG and EOG recordings, and static and dynamic perimetry. Similar measurements were taken at 4- to 6-month intervals over the last 20 months while her ornithine has been reduced to near normal plasma concentrations. No significant visual changes were noted for the first 13.5 months of reduced ornithine. However, in May 1979, the patient reported an improvement in her ability to dark-adapt when changing from high to low illumination. Measurements of dark-adaptation thresholds, taken within the following 2 months, confirmed this improvement, which has persisted and has been associated with progressive im-

provements in several tests of visual function.

Sensory dark-adaptation thresholds (10) measured for the left eye [oculus sinister (O.S.)] before the diet (1977) and currently (1979) (11) are shown in Fig. 1. The most striking improvement is the demonstration of a clear cone-rod break, not present before, at about 5 minutes of adaptation and a relative improvement of 1.5 to 2.0 log units in the final threshold after 40 minutes of adaptation (which still is approximately 1.2 log units higher than the mean normal value, although close to the 95 percent confidence upper limit of normal data). The inset in Fig. 1 shows final thresholds at 40 minutes of adaptation for both eyes over a 2-year period; The right eye [oculus dexter (O.D.)] has also improved, although less strikingly. This discrepancy between her eyes, which was also detected in other tests, probably reflects the results of an intraocular hemorrhage of the right eye that occurred 7 years ago. The improvement in dark-adaptation thresholds coin-

cided with a slight increase in the visual acuity of her right eye, which increased from a "counts finger (3 feet)" to 20/200 Snellen acuity over a 1.5- to 2-month period and has remained at that value until the present time. No improvement in the visual acuity of her left eye (20/30) has been detected.

The improvement in rod function was followed by an improvement in cone function, as measured in her color vision. By 20 months after treatment, the patient had a comparatively milder blue-yellow defect in her left eye, while the reduced central color vision of her right eye was expressed by a scotopic pattern in the D-15 test of Farnsworth (Fig. 2A). In comparison, prior to treatment her left eye showed a marked tetartanopic blue-yellow defect (12), while an anarchic pattern was obtained for her right eye in color discrimination and color confusion tests (Fig. 2B). Current D-15 test results show a normal response pattern in her left eye and an atypical red-green defect pattern in her right eye. The improve-

Fig. 1. Solid curves show dark-adaptation sensory thresholds for the left eye of the patient obtained prior to (June 1977) and 20 months after dietary treatment (September 1979). Dashed curve represents averaged dark-adaptation thresholds from a normal population ( $N = 189$ ); vertical bar represents 95 percent confidence interval for final adaptation threshold at 40 minutes. Inset shows the final adaptation thresholds for both eyes over a 2-year period; dotted band indicates 95 percent confidence interval.

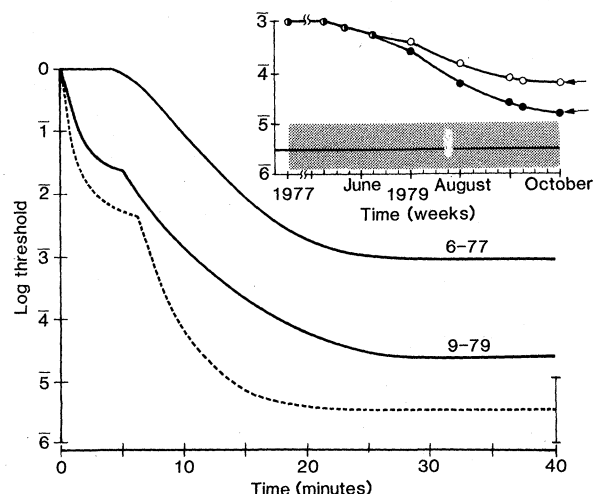
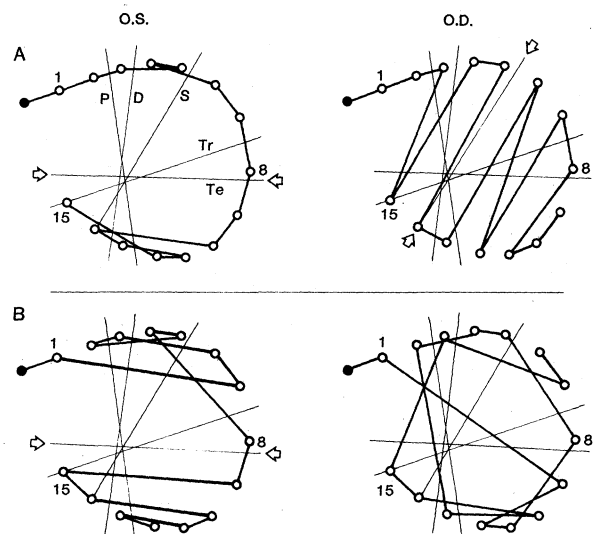


Fig. 2. Farnsworth-Munsell D-15 test patterns of the patient (A) after 20 months and (B) before dietary treatment. Numbers represent test cap number; closed circles represent reference cap. Arrows indicate axis of confusion errors. Protan (P), deutan (D), scotopic (S), tritanopic (Tr), and tetartanopic (Te) axes are indicated in the top left pattern. Normal response pattern for this color-confusion test consists of a sequential ordering of the caps from 1 to 15 (clockwise) with no errors or only minimal reversals (such as exchanging two adjacent caps).



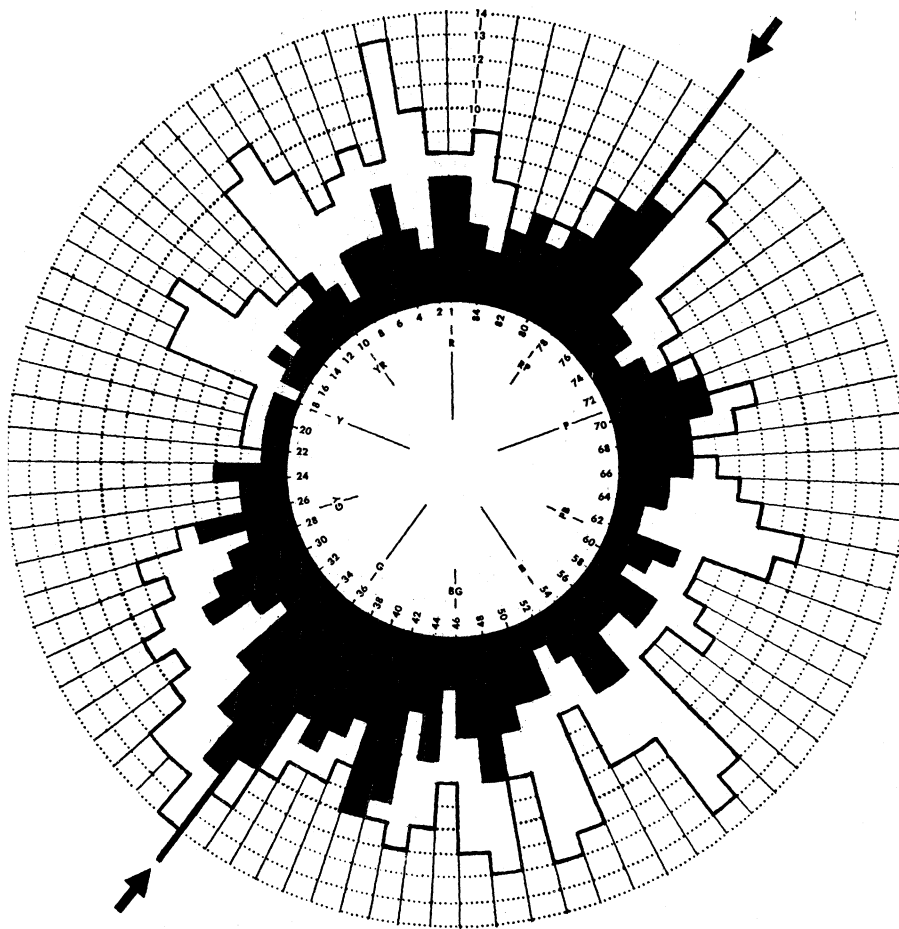


Fig. 3. Farnsworth-Munsell 100-Hue test patterns of the patient (left eye) before (open blocks) and 20 months after (closed blocks) dietary treatment. Arrows indicate tetartan axis. Normal response pattern for this color-discrimination test consists of a sequential ordering of the 85 caps with no errors or only minimal reversals.

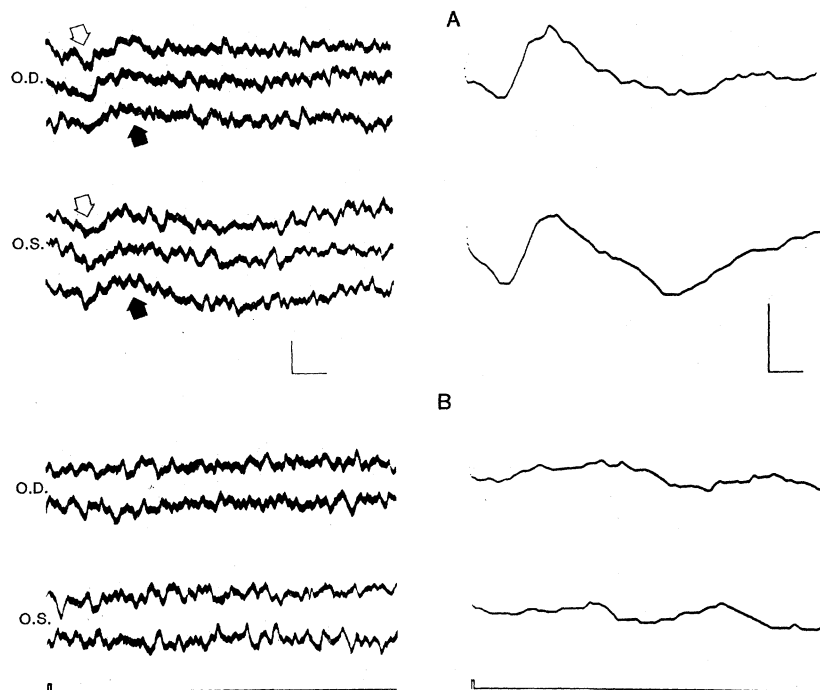


Fig. 4. Single-flash (left) and averaged (right) ERG responses to a white flash of maximum intensity in conditions of Ganzfeld stimulation and dark-adaptation after 20 months (A) and before dietary treatment (B). Calibrations: 50  $\mu$ V and 20 msec. Normal responses to these flashes have a similar waveform but peak amplitudes about ten times larger than those shown in (A). Open arrows point to a-wave potentials, closed arrows to b-wave potentials.

ment in her color discrimination was expressed in the 100-Hue test by a reduction of the total number of errors and the reorganization of the response errors from a rather anarchic pattern to a bipolar tetartan-like pattern (green/red-purple axis). This improvement again was more noticeable in her left eye (Fig. 3) than in her right eye. Dynamic and static perimetry have also shown improvement in her visual fields. Over the past 2 years there has been a roughly concentric, 10° radial expansion in the visual fields of both eyes for Goldmann targets III<sub>4e</sub> and IV<sub>4e</sub> (13). No significant changes in her fundal appearance have been observed.

The ERG which was extinguished prior to treatment has also improved and now shows measurable rod and, to a lesser extent, cone responses to Ganzfeld stimulation. Figure 4A shows single flash (left) and average (right) ERG responses to strong white flashes (14) presented when the patient was fully dark-adapted. These responses were recorded after 20 months of dietary treatment. For comparison, Fig. 4B shows ERG recordings obtained with the same conditions of stimulation before treatment. Although still subnormal in peak amplitude, rod-dominated potentials from either eye have significantly increased in amplitude and can now be detected in single-flash responses to white and blue lights. Despite the differences in the final dark-adaptation thresholds between the left and right eyes, these Ganzfeld-ERG rod responses generally show waveforms of similar amplitude, time course, and implicit time for both eyes. With our techniques, light-adapted ERG cone responses (not shown) can currently be observed only in averaged recordings, mostly from the left eye. Despite their subnormal amplitude, these cone responses have also improved, as they had been extinguished before treatment. No statistically significant changes in the light-peak/dark-trough ratio of the EOG have been observed during the last 20 months; this ratio remains subnormal (range: 120 to 136 percent O.D., 116 to 126 percent O.S.).

The improvement in visual function after reduction of ornithine accumulation suggests that the pathophysiology of GA may involve (i) either a direct toxic effect of ornithine or (ii) a deleterious effect of a metabolic alteration occurring secondary to the hyperornithinemia, or both. Since hyperornithinemia due to other causes is apparently not associated with chorioretinal degeneration (15) deficiency of OAT may be necessary for manifestation of the pathophysiologic effect. Baich and Ratzlaff have found that

the specific activity of OAT in the pigment epithelium of adult mammals and birds is much higher than that in other tissues such as retina, liver, or kidney (16). This finding suggests that OAT has an important function in the normal physiology of the pigment epithelium, and consequently, the retina. Given the major role of the pigment epithelium in the renewal process of photoreceptor outer segments (17, 18) and the different renewal rate between rods and cones (17), pigment epithelium dysfunction due to OAT deficiency could conceivably explain the differential effects of GA upon rod and cone function and their differential improvement by a low arginine diet.

Our findings in a clinically typical GA patient demonstrate significant improvements in visual function 13.5 months after the reduction of plasma ornithine to near normal levels. Similar improvements, limited to visual acuity and visual field, are suggested in the preliminary findings of McInnes *et al.* (19). The improvement in our patient after 13.5 months of near normal plasma ornithine concentrations suggests that it may be possible to halt the progression or even partially reverse some of the alterations by means of a low arginine diet. Although confirmation will require continued follow-up of this patient as well as dietary treatment of other GA patients, the results indicate that such treatment may be considered for this progressive disease.

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10. Measured with a modified Goldmann-Weekers adaptometer with corrective optics following a 3-minute period of Ganzfeld light adaptation (515 cd/m<sup>2</sup>).
11. This improvement has been documented most recently in June 1980.
12. Tetartanopic response pattern in D-15, 100-Hue, and HRR color vision tests. At the initial stages of improvement, this eye showed two neutral points, one at about 460 nm and the other at about 570 nm, when examined with a saturation discrimination test. At the present time, however, this eye shows an elliptical enlargement of the neutral area (maximal and near-maximal desaturation zone) along the tetartan confusion axis.
13. Measured in a Goldmann perimeter with corrective optics to provide best possible visual acuity; targets III and IV are 4 and 16 mm<sup>2</sup>, respectively. Measurements were obtained by the same person and in good conditions of fixation.
14. The ERG responses were obtained with a contact lens electrode (Burian-Allen) differentially connected with an a-c amplifier (gain,  $\times 1000$ ; band pass, 1 to 300 Hz) and displayed on a storage oscilloscope. A forehead or earlobe electrode grounded the patient. Ganzfeld stimulation was used throughout the study according to a modified version of the stimulator described by R. D. Gunkel, D. R. Bergsma, and P. Gouras [*Arch. Ophthalmol.* 94, 669 (1976)]; the highest setting of the photostimulator unit (Grass PS22) was used to discharge the flash tube. The patient was fully dark-adapted for 1 hour, and the elec-

trodes were mounted under very dim red-light illumination; one eye was tested at a time, the fellow eye being carefully occluded to avoid light-adaptation and to reduce blinking to the flashes. Light-adaptation was obtained with a steady white (2830 K) background of 2.84 log trolands. Averaged responses were electronically summed with a signal averager (Nicolet 1072) (64 trials; 391  $\mu$ sec per bin, 512 bins) connected to the vertical plates of a slave cathode-ray tube (via a cathode follower) displaying single-flash responses.

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20. D.V. is an investigator in the Howard Hughes Medical Institute. We thank our patient Z.M.F. for her active cooperation during this study. Supported in part by grant RR-52 from the General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health, and by grant EY-02948 from the National Institutes of Health.

7 May 1980; revised 6 August 1980

## Relation of Mammalian Sperm Chromatin Heterogeneity to Fertility

**Abstract.** *Flow cytometry of heated sperm nuclei revealed a significant decrease in resistance to in situ denaturation of spermatozoal DNA in samples from bulls, mice, and humans of low or questionable fertility when compared with others of high fertility. Since thermal denaturation of DNA in situ depends on chromatin structure, it is assumed that changes in sperm chromatin conformation may be related to the diminished fertility. Flow cytometry of heated sperm nuclei may provide a new and independent determinant of male fertility.*

The factors that affect male fertility are poorly understood, although it is known that sperm cell count, shape, and motility are important. Since sperm nuclear morphology is related to chromatin condensation and other nuclear phenomena occurring during spermiogenesis, we hypothesized, as have others (1), that misshaped sperm nuclei have an altered chromatin structure. Furthermore, since the resistance of in situ DNA to thermal denaturation is related to counterion and protein interactions with DNA (2), it seemed likely that an altered chromatin structure would be reflected in an abnormal DNA denaturation profile. We report here that not only does the in situ DNA of misshaped sperm nuclei have a significantly decreased resistance to thermal denaturation, many morphologically normal nuclei derived from subfertile donors are also abnormally susceptible to in situ thermal denaturation of their DNA. We suggest that the structure of sperm chromatin, as reflected by its sensitivity to thermal stress, may be an additional determinant of fertility.

Resistance of sperm nuclear chroma-

tin to heat denaturation was determined by heating isolated sperm nuclei (3) and then staining with acridine orange (AO) prior to analysis by flow cytometry. The differential staining of native versus denatured DNA is due to the metachromatic properties of AO; when intercalated into native double-strand DNA the dye fluoresces green ( $F_{530}$ ); when stacked on single-strand DNA it fluoresces red ( $F_{600}$ ) (2). Therefore, the level of DNA denaturation in situ can be determined by measuring the ratio of red fluorescence to total nuclear fluorescence (red + green); this ratio varies experimentally from about 0.1 (undenatured) to 0.9 (highly denatured) and is termed  $\alpha_1$  (2). Figure 1 shows the data for sperm nuclei from a bull of high fertility and from one of low fertility. Note that the position of the main cluster of nuclei from the fertile bull was changed very little by heat, indicating that the DNA was resistant to thermal denaturation. The peak value of the  $\alpha_1$  histogram (inset) (2) is low, near 0.1. In contrast, a very marked difference in AO fluorescence was induced by heating the nuclei