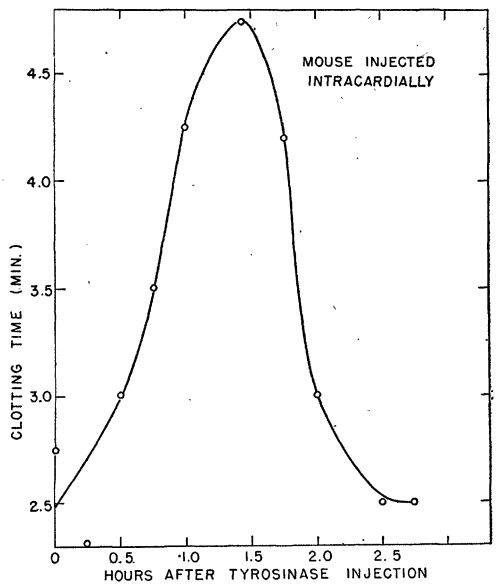

FIG. 2. Effect of 0.2 ml tyrosinase, injected intracardially in a 25-g mouse, on the clotting system of the blood. Blood samples were removed at successive intervals from the tip of the tail for measurement of clotting time.

blood not only clotted slowly but never produced as firm and rigid a clot as did normal blood.

Typical changes in clotting time resulting from the injection of 0.2 ml tyrosinase into a 25-g mouse are shown in Fig. 2. Although the clotting time is about doubled within 1.5 hr after the injection, the clotting mechanism returns to normal in 3 hr. In a series of experiments the clotting time showed a two- to four-fold increase within 1.0–1.5 hr after the tyrosinase was injected. The restoration of a normal clotting system in the treated mouse was often delayed for 8 or even 24 hr in some experiments. Animals which showed the greatest effect of tyrosinase on the blood often appeared sick and occasionally died in 24–48 hr. In control animals which were injected with boiled tyrosinase the clotting system was unaffected during the 8 hr of the experiment.

The problem of immunization of mice to tyrosinase was investigated by using a single animal for a series of injections over a period of 1–2 months. Results with several animals clearly show that injections of tyrosinase cause the production of antibodies which inhibit the enzyme *in vivo* (*7*). For example, in one mouse the clotting time increased to 11 min when tyrosinase was injected the first time; 15 days later when it was injected again the increase was up to 5 min, and 8 days later a third injection did not noticeably change the clotting time from the normal value of 2.6 min.

These studies show that tyrosinase rapidly inactivates the clotting system of plasma, whole blood, and of circulating blood of the mouse. In the living animal the clotting system returns to normal after several hours. Experiments are in progress to determine which components of the clotting system of blood *in vivo* and *in vitro* are inactivated by tyrosinase and to determine whether the eventual restoration *in vivo* of the system to normal represents a reversal of the inactivation or a replenishment by the blood-synthesizing organs of the inactivated components.

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The Nonsurgical Treatment of Cataract[1]

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Because of the high incidence of cataracts and the hazards associated with their surgical removal (disregarding psychological and economic factors), a nonsurgical treatment of cataracts has been sought by ophthalmologists for many years. At one time it was thought that the administration of bovine lenticular extracts might be of therapeutic value (*1*), but this was not established (*2*). Subsequently, and because of the differences that were noted between lenticular extracts from fish and the proteins of mammalian lenses (*3–5*), the possibility of fish lens proteins being of therapeutic value was considered.

In November 1950, experimental work was started to study the ocular changes in rats subjected to high dietary stresses. The results, as yet unpublished, cover more than a year's work on the lens changes in rats under controlled conditions. Groups were large enough (24 or more) to compensate for individual biological differences. Selected groups of individually caged rats were placed under a physiological stress such that changes might, and did, occur in the eyes. These eye changes were ophthalmoscopically observed and critically described each week. Concurrently, blood and urine chemistry, employing microtechniques supplemented by paper chromatography, was carried on. Initial experiments were performed on rats 6 weeks of age; but before the work was completed, not only were all age groups studied, but stresses applied to females at time of breeding were finally evaluated, in order to differentiate between the effect on the offspring during fetal life and during lactation, by examining the eyes of baby rats at weaning. These experiments, which involved many hundreds of rats whose eyes were examined ophthalmoscopically weekly, ultimately made it possible to induce cataracts

[1] The patients were treated at the office of Dr. Ginsberg, 705 New Jersey Ave., Brooklyn, N. Y.

at will, the degree of opacification being a function of age or stress, or some combination of age and stress. Obviously, the development of a bio-assay technique will be of tremendous value in future studies.

Recently, in a large series of experimental animals, controlled induced cataracts were caused to regress to disappearance by the parenteral administration of fish lens protein extracts. Such cataracts could not again be reinduced experimentally with the original stress if, concurrently, fish lens protein extracts continued to be administered. As a result of these experiences, we decided to administer a fish lens protein extract to human cataractous patients. The results obtained have to date been so promising that it was thought advisable to make this preliminary report.

The lenticular extracts are prepared by comminution of the lens tissues, their extraction with a physiologically compatible solvent, the removal of insoluble residues, and the sterilization of the final solution. Total protein concentrations are less than 2% as determined by the usual Kjeldahl procedures: the pH is approximately 7.4; and although several thousand injections of the fish lens extract have been administered, no allergic reactions have been observed. Empirically, a dosage of 1.0 ml administered thrice weekly has been standard with all cases discussed in this preliminary report. Thirty or more times this dose (i.e., 0.5 ml/kg body weight for rats, given every day for several weeks) has produced no discernible untoward effects.

The first group, totaling 14 patients, has now had four or more subcutaneous injections of this extract. The degree of visual impairment has ranged from patients who had been unable to carry out their normal household duties for several years, or had been unable to move about the streets unattended, to patients manifesting only visual fogging. In all cases, after four injections patients have reported subjective improvement. Objectively, after six or more injections administered three times weekly, a diminution of lens opacities has been observed ophthalmoscopically (Figs. 1 and 2). As a result of this treatment in several cases who have had 30 injections, the progress has been from a completely incapacitated individual to one with practically normal visual acuity. Most of the patients have been between 60 and 70 years old, and in most cases the cataracts have been typical senile opacities. Several of the patients had cataracts associated with diabetes and were hypertensive, or arteriosclerotic, or had combinations of these conditions. In all cases there has been a clearing of lens opacities, and retinal detail, which previously could not be seen, can now be studied with the ophthalmoscope.

Concurrently with these preliminary clinical trials, biophysical studies are being made that indicate tremendous differences between the proteins of the lens of the mammalian eye and those of fish lenses. The ultracentrifuge and the electrophoretic apparatus have been employed in these studies, and for the first

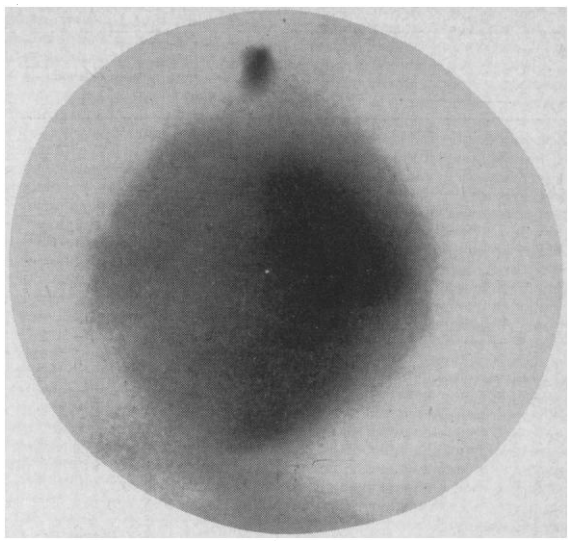

FIG. 1. Patient L: original ophthalmoscopic photograph of appearance of cataract in right eye.

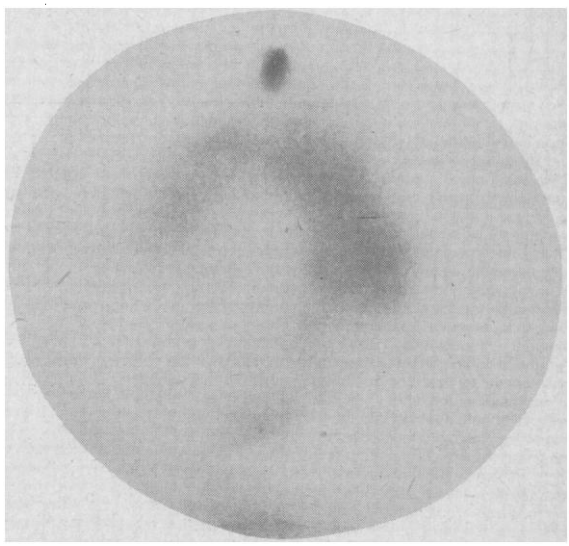

FIG. 2. Patient L: ophthalmoscopic photographic appearance of right eye after about two month's treatment.

time information on the distribution, molecular weight, and electrophoretic motilities of lenticular proteins has been obtained. Essentially the pattern that in the past was applied to blood plasma studies has been followed. It is expected that not only will the components of fish lenses be fractionated and characterized in the customary terms of electrophoretic purity, molecular weight and dimensions, configuration, electrical charge, etc., but that these fractions will be evaluated, utilizing the rat bio-assay already mentioned, in terms of biological activity. They will be published as soon as the data can be correlated, and the necessary calculations made.

Results in an additional 12 cases, selected more or less at random from nearly 100, which are to be the

TABLE 1

Patient	Age	Visual acuity		Dates (1952)	No. injections
		O.D.	O.S.		
Mrs. S. W.	34	Light and dark	20/20–3	Feb. 27	
		20/40	20/20–3	July 9	40
Mr. S. S.	64	20/100	20/200	Feb. 28	
		20/30	20/50	Apr. 29	29
Mrs. E. D.	54	Light and dark	20/100	Feb. 24	
		20/100	20/30	May 21	40
Mr. M. F.	67	20/60	Amblyopic	Jan. 9	
		20/15		Mar. 6	22
Mrs. G. R.	49	20/150–1	20/30–2	Feb. 18	
		20/40–3	20/15–3	Apr. 29	30
Mrs. S. J.	54	20/200	20/150	May 12	
		20/200	20/40+	July 16	28
Mrs. M. M.	60	Aphakial	20/150	Jan. 22	
			20/60	Mar. 19	30
Mrs. C. P.	70	20/70	20/80	Apr. 23	
		20/30	20/30	July 9	36
Mr. P. de P.	39	20/50–2	> 20/200	Apr. 3	
		20/20+	20/100	June 25	41
Mrs. I. L.	57	20/70	20/30	Apr. 30	
		20/40	20/30	May 28	12
Mrs. J. A.	67	Fingers at 5 ft	20/80	May 21	
		20/100	20/40	July 16	26
Mrs. P. B.	70	20/80	20/60	Jan. 2	
		20/20–2	20/20–1	Apr. 17	34

subject of an extensive and more detailed clinical report, are shown in Table 1. No attempt is made to amplify the mitigating factors that affect visual acuity, such as retinopathy, which is not discovered until a clearing of the lens permits a detailed examination of the fundus; nor are the sketches and written descriptions of the ophthalmoscopic appearances of the opacities included. These data, as well as all other pertinent observations regarding coexisting pathology, such as diabetes, hypertension, etc., and subjective changes reported by the patients, have been a normal part of the critical evaluation procedures employed. For the purpose of this brief and preliminary paper, a record of the initial and final visual acuities, together with the ages of the patients and the number of injections received, should answer that first and most important question that will be asked in regard to this nonsurgical therapy of cataract: "Is the patient's vision improved?"

Another clinical series involving approximately 50 patients is being observed and studied under conditions of the most critical scrutiny, and these results will be submitted for publication as soon as available. In view, however, of the promise that this therapy has shown so far, this report is submitted in order that those interested in the subject may be advised of the work that is in progress.

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Observations on a Chlorophyll-Deficient Strain of *Chlorella vulgaris* Obtained after Treatment with Streptomycin

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It has been demonstrated that streptomycin brings about a loss of chloroplasts in the flagellate *Euglena* (*1*) and in the seedlings of certain Gramineae, and that it causes the loss of color in crown gall tumors of carrot (*2*). Therefore, research was recently initiated to determine whether streptomycin would induce the loss of chloroplasts in algae other than *Euglena. Stichococcus bacillaris, Scenedesmus dimorphus,* and two species of *Chlorella* (sp. and *vulgaris*) were investigated. All these species are capable of heterotrophic growth in the dark, with glucose as a carbon source.

Vegetative cells of all these algae were treated in tubes containing Maerten's solution ($Ca(NO_3)_2 \cdot 4 H_2O$, 1.0g; $MgSO_4 \cdot 7 H_2O$, 0.2g; K_2HPO_4, 0.2g; 1 ml of trace element solution [*3*]; and distilled H_2O, 1000 ml), 0.5% glucose, and four concentrations of streptomycin sulfate (500, 2500, 5000 and 7500 μg/ml). One