BY LINDA GARMON

Giving NMR the Eye

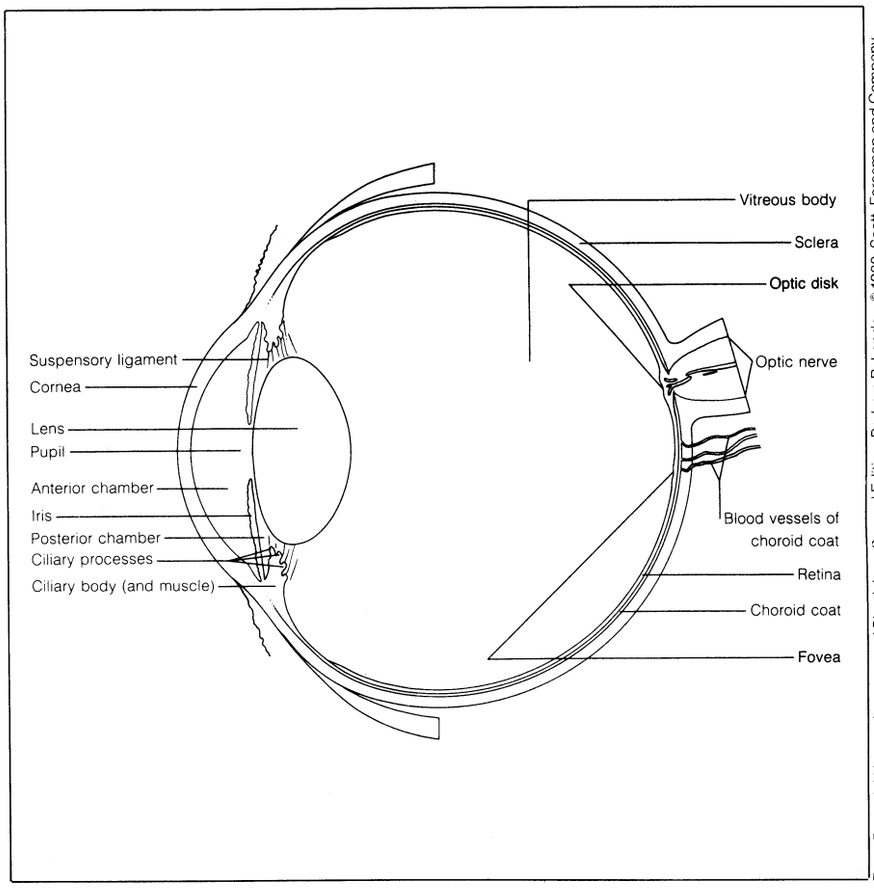
Determining the chemical events that precede formation of cataracts is the new job of an old analytical technique called nuclear magnetic resonance spectroscopy

Just as clear photography depends on precisely focusing incoming light rays onto film, clear vision depends on focusing beams of light onto the retina. Consequently, when the normally crystalline lens — a component of the eye's light-focusing apparatus — loses its transparency there is a breakdown in the "biologic camera." This process, known as cataract formation, leads to partial or total blindness.

While cataractous lenses often can be removed and effective vision restored via use of special eyeglasses, researchers hope ultimately to be able to reverse or even prevent their formation. Success on this front, though, depends on a thorough understanding of the chemical events involved in cataract formation, and thus far, technical limitations have kept this beyond the ophthalmologist's grasp. Now, however, scientists have transformed an old analytical technique into a tool that not only is helping to unravel the molecular mysteries of cataract formation, but also is illuminating serious flaws in the conventional test models of this phenomenon.

That tool is nuclear magnetic resonance (NMR) spectroscopy, and as its name implies, it gathers information from atoms whose nuclei are magnetic. All major particles in a nucleus — that is, protons and neutrons — have magnetic moments, explains Thomas Glonek of the Chicago College of Osteopathic Medicine. These miniature magnets form pairs, a north-south teaming with a south-north, to leave the overall magnetic moment of the nucleus nil. But in many nuclei, an unpaired neutron is leftover, and that particle causes the entire atom to be magnetic, says Glonek, who, along with Jack V. Greiner and colleagues, is using the properties of such atomic magnets to probe lens chemistry.

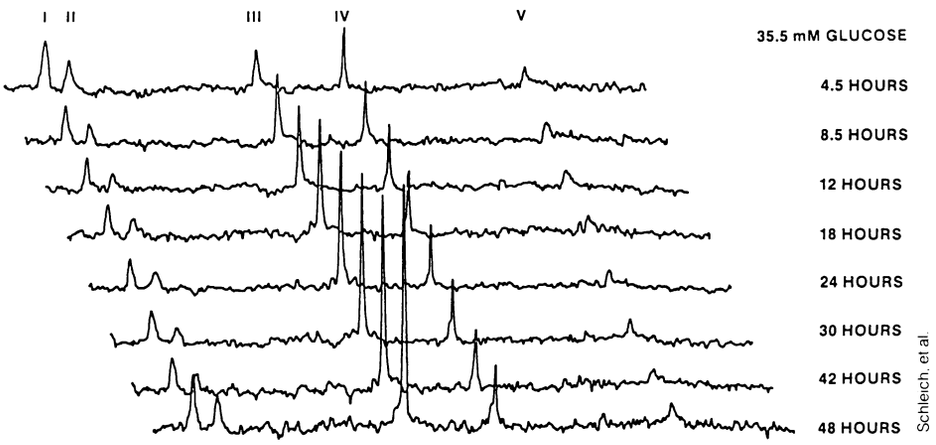
Using NMR technology, the magnetic atoms in a sample first are allowed to align themselves in a magnetic field and then are knocked out of alignment with low-energy radio waves. The energy that a particular atom absorbs to disalign itself produces a characteristic signal that is recorded as a printed peak on an NMR spectrum. The position of the peak on a printout helps identify the molecule on which the atomic atom sits, and the area under the peak represents the quantity of the molecule present in the sample.



From *Essential Human Anatomy and Physiology*, Second Edition, Barbara R. Landau, © 1980, Scott, Foresman and Company.

In the early days of NMR, printouts consisted of peaks from the magnetic isotope of hydrogen (H-1), which served only to help chemists determine structures of molecules in solids and solutions. At that point, because the hydrogen spectrum from a living system usually shows nothing more than a signal for water, NMR spectroscopy was virtually useless on biologic samples. (H-1 NMR *spectroscopy* differs from its descendant H-1 NMR *imaging* — a picture-producing analytical technique that some researchers believe shows cancerous tissue-detecting potential [SN: 6/9/79, p. 380].) The technique was slow to find biochemical applications, Glonek explains, because signals of atomic nuclei more relevant to living systems were too weak to detect with early NMR technology. Eventually, though, with the aid of high-powered magnets and more sensitive spectrometers, researchers began probing biochemically relevant nuclei — those from phosphorous-31 (P-31) and carbon-13 (C-13) — in systems such as red blood cells and muscles. Thus began the NMR probe of complete biological components, an area of research that recently embraced analysis of the chemical events that may initiate cataract formation.

Previously, the study of eye chemistry meant analytically sifting through many ground up lenses in hopes of extracting just one small piece of information. Now,



This series of C-13 NMR spectra illustrates the metabolism of a single rabbit lens in a high glucose medium. The peaks represent carbon from various compounds: I and II = two glucose isomers; III = sorbitol; IV = a control compound; and V = lactate.

using the NMR approach, "real-time" data regarding lens metabolism can be obtained without destroying the removed lens. "This kind of research is beautiful," says Greiner, "because you can take a lens and study it biochemically [with NMR] and then further study it anatomically, which involves a destructive analysis." Before NMR entered the picture, he says, the same lens could not be used for both analyses, because both were destructive techniques. Moreover, maintaining the chemically active lenses necessary for this approach is fairly simple because lens tissue *in vivo* is not directly fed by blood vessels; instead, it receives its nutrients from a fluid that can be easily mimicked in the laboratory. As a result, animal lenses can be removed intact and placed in a culture that in turn can be experimentally manipulated to test theories of cataract formation.

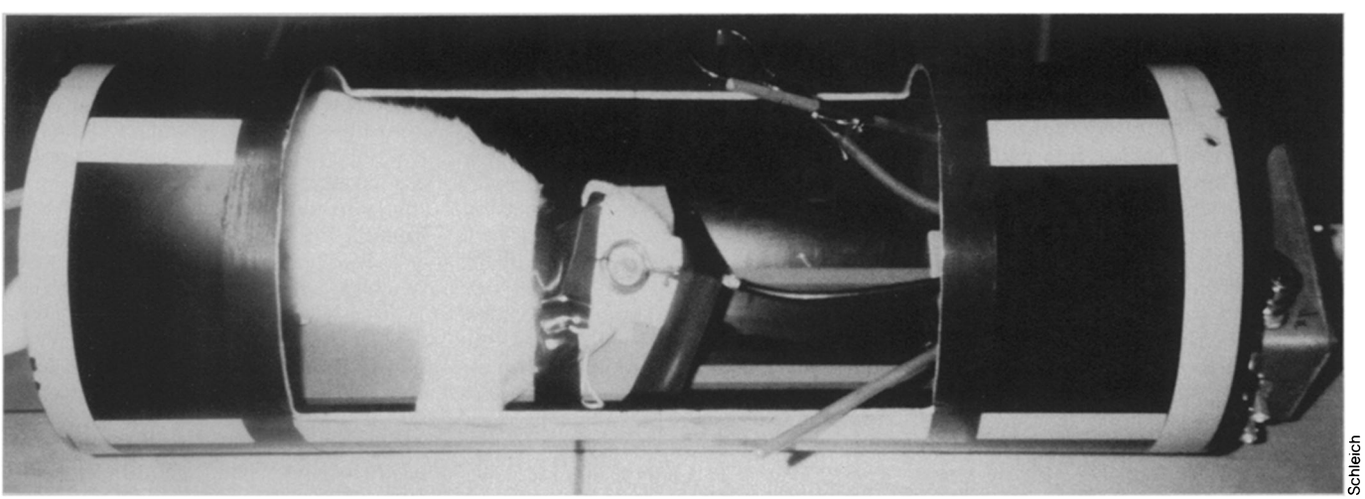
For example, Greiner, Glonek and colleagues have investigated a theory regarding formation of the rare hypoglycemic cataracts — clouded lenses that result from glucose deficiences. These can develop in small children whose blood glucose levels are depressed due to habitually missed meals, Greiner says. Researchers have theorized that such cataracts result from a shut-down of a cellular "pump" run on the chemically stored energy that is released when the molecule adenosine triphosphate (ATP) splits into adenosine diphosphate (ADP) and inorganic phosphate (P_i). The ATP needed to run this pump — which is responsible for shuttling sodium and potassium ions back and forth across cell membranes — is generated in a complex metabolic pathway that uses glucose as fuel. If the lens lost control of the pump system due to lack of sufficient fuel, the theory states, potassium and sodium ions would accumulate. Then, because of an osmotic effect, water would move into the lens, swelling its tissue and reducing its transparency.

To test this theory, Greiner and associates used P-31 NMR spectroscopy — which measures the phosphorous-containing components of a sample — to analyze lenses that had been removed from rabbits and placed first in "control" culture and then in glucose-deficient media. The resulting spectra revealed that five hours after glucose removal, the amount of phosphorous constituting ATP molecules fell from 47 to 10 percent of the total lens phosphorous. A concomitant rise of P_i also was evident. In addition, the Chicago group noted that as ATP levels fell, lens pH rose — an indication that ions were amassing. These results — published in the November INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE — support the hypothesis that hypoglycemic cataractogenesis is initiated when a depleted ATP supply compromises the ability of the lens to pump ions.

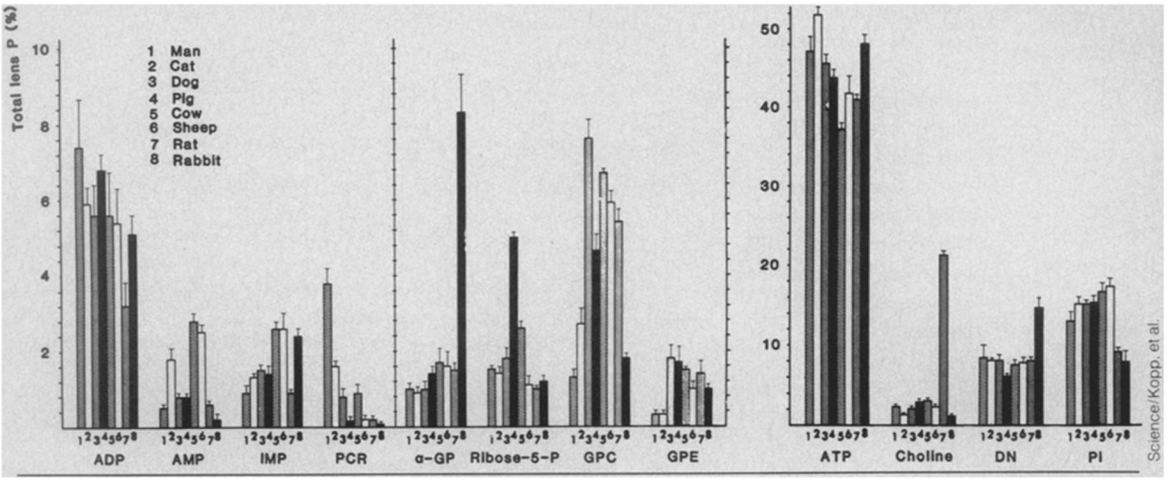
That study laid the NMR-and-cataract-chemistry groundwork for the Chicago team. They next went on to discover that certain accepted classical models in cataract research may be flawed.

Greiner and cohorts were using NMR spectroscopy in conjunction with a classic experimental model employed to mimic cataract formation in galactosemia patients. Galactosemia is a rare, inborn metabolic error. Individuals with this disease have a decreased ability, due to a missing enzyme, to make glucose from galactose — the simple sugar formed from the splitting of the milk carbohydrate lactose. The consequent excess galactose somehow seeds the growth of cataracts.

Conventional models used to probe the precise chemistry behind galactose-induced cataract formation involve one of



An anesthetized rabbit is being readied for NMR measurements of eye tissue. Although the radio waves to be administered are a form of radiation, researchers presume they will pose no significant risk to living systems because they are of the long wavelength, low-energy form. Still, research on that subject has been limited and thus far is non-conclusive.



Bar graphs illustrate the interspecies variations in phosphorous-containing metabolites of the crystalline lens.

two methods: feeding massive amounts of galactose to test animals and then analyzing removed lenses or removing the lenses first and then assaulting them with high levels of the sugar. Formation of cataracts has been observed in both cases. In both models, though, the enzyme that is absent in galactosemia patients is present. The presumption is that even with the enzyme present, these systems cannot handle in the normal metabolic way the entire masive amount of galactose that is administered in test situations; whatever method the systems use to handle the overload imitates the galactosemia lens chemistry and, in so doing, causes cataracts. Researchers using these presumptive models consistently conclude that conversion of galactose to a sugar alcohol plays a role in galactosemia cataract formation.

Now, however, results of the Chicago team's study question the validity of these conventional galactosemia models. In the normal metabolic route, galactose is converted to glucose, which in turn is fed into a metabolic pathway called glycolysis. When Greiner and colleagues — repeating the classical experiments — assaulted lenses with excess galactose, NMR spectra over a 20-hour period indicated a 300 percent increase of a phosphorous-containing chemical component of that glycolytic pathway. While this enormous surge in a component of glucose metabolism would not be expected to play a role in *true* galactosemia chemistry, it nonetheless must be considered a cataract-causing suspect in the test models. This suggests that the cataract-causing agent in classical galactosemia models may, in fact, bear no resemblance to the real-world culprit. "Any chemical that increases by 300 percent has to be considered a stress and must be taken into account," says Chicago team member Stephen J. Kopp, whose study will be published in the May INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE and presented that same month at the Association for Research in Vision and Ophthalmology meeting in Sarasota, Fla.

As in their earlier study of hypoglycemic cataracts, Greiner and colleagues used P-31 NMR spectroscopy to probe the chemistry of classical galactosemia models. Another form of NMR relevant to cataract chemistry, C-13 NMR spectroscopy, is dominating the study of lens bioenergetics at the University of California at Santa Cruz.

There, Thomas W. Schleich and colleagues are using C-13, a rare, nonradioactive isotope of carbon, to investigate one of the most common types of cataracts — that which occurs in persons with diabetes mellitus. The incidence of cataract-formation among persons with the disease is about five times greater than that in the general population, Schleich says. Researchers believe that, at least in animals, the so-called "sugar cataracts" form because the lens cannot handle the entire excessive amount of glucose characteristic of diabetes by driving it into the normal metabolic pathway of glycolysis. Instead, some of the glucose overload is converted to the sugar alcohol sorbitol, which accumulates in the lens and causes an osmotic pressure. Water then moves into the lens to relieve the pressure, and transparency is reduced.

Schleich and colleagues "observed" this dynamic event in rabbit lenses placed in media with high levels of C-13-labeled glucose. NMR spectra accumulated over a 48-hour period indicated that the glucose stress led to a three-fold increase in sorbitol.

The highlight of Schleich's study — presented in February at the Biophysical Society meeting in Boston and accepted for publication in INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE — is not the observed increase in sorbitol, but rather the fact that each NMR spectrum was taken on a single lens. To date, most NMR techniques require three or four lenses stacked in a test tube, Schleich explains. Developing an effective one-lens technique, he says, is the first step toward a truly non-invasive analysis — that is, using NMR spectroscopy to analyze lenses in the eyes of living animals.

Schleich is studying the possibility of a purely non-invasive NMR analysis of rabbit lenses. The rabbit first is anesthetized; then, a copper coil — which applies the low-energy radio waves — is placed around one of its eyes; finally, the animal is situated in the magnetic field. Preliminary tests are being conducted in England, where Oxford Research Systems Ltd. manufactures a special powerful magnet with a bore (the center hole of the magnetic ring) large enough to fit the whole rabbit. "If this thing ever takes off, perhaps some time in the future this might — and I emphasize might — be developed into a technology that can analyze lenses in humans," Schleich says. "It might be pie-in-the-sky," he says, "but we gotta try."

Before non-invasive human analysis comes to cataract research, though, the NMR already will have caused a revolutionary change in the field. Traditionally, researchers studying lens chemistry have relied mostly on rabbit and rat lenses to generate data. Both types of lenses are convenient to obtain and inexpensive. This reliance on rabbit and rat lenses soon may change, however, in light of NMR research results reported by Kopp, Glonek and Greiner in the March 26 SCIENCE.

The Chicago researchers used P-31 NMR spectroscopy to compare the chemical differences among humans and various animals. (The human lenses were removed within two hours after death of persons 24 to 30 years of age.) "Overall results indicate that the cat and dog lenses most closely resemble the human lenses in that they have the fewest number of significant metabolite differences," Kopp and associates say. "Generally," they report, "the rank order of the lens metabolism from the most to least similar to human is as follows: cat ~ dog > pig > rat > sheep > rabbit > cow."

"This is going to cause people to reassess the value of any results they've obtained using rabbit and rat lenses," Kopp says. "This interspecies variation in mammalian lenses is not something people could have looked for easily in the past," he says. "The NMR permits one to evaluate the differences quickly."

Says Kopp, "We're very proud of this study. It shows the power of NMR in lens research." □