Arch. Soc. Amer. Oftal. Optom. (1970) - 7 - 287 -

A CHALLENGE TO MEN OF VISION, THE CRYOOPHTHALMOLOGISTS

BY

B. LUYET, M. D.

Madison - U.S.A.

When one surveys the behavior of human beings during the millenia that they spent occupying the earth, he is struck by the fact that, though they were submerged in a material world, and had to fight continuously for material subsistence, they found ways of widening their horizons beyond the purely materialistic. They let themselves be inspired by ideals, such as the determination of helping their fellow men. Among the many manifestations of such ideals we shall, on this anniversary of the foundation of the Sociedad Americana de Oftalmología y Optometría, consider together some of the humanitarian activities in the preservation of the precious treasure of vision.

I felt proud of belonging to the human race when I was shown a picture of some hundred hospital sisters who together pledged to donate their eyes to an eye bank after their death (Sisters of St. Mary, in St. Louis, Missouri, 1960). I have a similar feeling when I consider the dedication of thousands of ophthalmologists throughout the world who devote their lives to the improvement of man's vision, and, in particular, when I consider their efforts, during the last few decades, to find means of permitting a brotherly exchange of the organs of vision and, concurrently, means of preserving these organs for future exchange. Seen in that light, the rapid development of Cryoophthalmology in recent years, to which Dr. Barraquer made outstanding contributions, becomes an event which deserves mention in the history of the world. I would like, now, to examine with you the close natural connection between these recent developments in ophthalmology and some of the great events in the evolution of the world. In the whole, our survey might be entitled: A Meditation on the Marvels of the World of Light.

Let me point out first the similarity between the tool we are using in our attempts to grasp the mysteries of the world —that is, human intelligence— and the tool our body uses to get material information about its surroundings —that is, the eye. Both use the method of drawing pictures of things. Both may be said to be picture mills. Furthermore, the development of our intelligence is, to a large extent, the result of the activity of our eyes. It may even be said that our intelligence is the prolongation of ocular vision. We think by means of pictures. Let us now apply this very method of thinking to our survey of the great events to be described. That is, we are going to glance at the history of the world in a series of pictures (there will be seven of them). This will constitute the first part of the present paper. The second will be devoted to the challenge announced in the title.

PART I

A Glance At The History Of The World With Particular Reference To The Role Of Light

Picture Nº 1

The World of "Intensely Active Bodies"

What strikes our intelligence as being one of the primordial facts in the universe is the existence of billions of bodies (the stars) which are characterized by a very intense activity; their constituent particles are continuously bombarding each other at immensely high speeds. This is the same as saying that they are very hot, for, temperature is nothing else but the rate of motion of the constituent particles. We have a fair notion of that intense heat in the case of our closest star, the sun.

The picture of these intensely active bodies represents what we actually see either with our unaided eyes or through telescopes, when we gaze at the stars at night. But, while our eyes and telescopes see only a part of the spectacle at a time, the eyes of our imagination draw the whole picture.

To handle pictures, we put them in frames. In the same manner, our mental pictures of the world are set in a mental framework, the concepts of space and time. Our understanding of the universe is entirely based on space and time. To use the expression of some philosophers, the space-time perception represents an "a-priori form of the mind".

To recapitulate, our Picture No. 1 is that of millions of suns, dispersed over an inconceivably large space, and lasting for a stafferingly long time. They are, by their nature, continuously flooding the universe with light.

Picture Nº 2

The World of Light and of Radiations

God said: "Let there be light" and there was light. This momentous event, the creation of the world of waves, was the starting point of some of the greatest happenings in the history of the universe. The mere fact of the existence of hot bodies means the emission of radiations.

Let us try to visualize the world at that stage, long before there was any human being, long before there was an earth. The numerous suns were continuously flooding space with beams of light, or rather with beams of all denominations, from the shortest X-rays and gamma rays to the longest radiations. Imagine, that is, see with the *eyes* of the imagination, that interplay of waves crossing the universe in all directions at a terrific speed (300,000 kilometers per second) and doing it for billions and billions of years.

Picture Nº 3

The World of "Bodies of Sluggish Activity"

There were in the Universe other bodies, which, from the cosmic point of view, are dense aggregates of molecules, sluggish in their movement, in a condition which we call cold. One of these bodies, the earth, was rotating around the sun and continuously flooded, for some two billions of years, by the rays of the sun.

PICTURE Nº 4

The World of Life

After such a flooding, or to put it in other words, after that kissing for billions of years between the world of waves and the world of cold bodies, and possibly as a result of that kissing, there developed at the surface of the earth some aggregates with very particular properties, among them that of duplicating themselves. This is the world of living beings, with all its wonders .

PICTURE Nº 5

The Collaborative Action of Living Matter and Light

Some of the living beings underwent one of the most mysterious and most remarkable processes. Flooded with light for half a billion years (or rather for half that time, since the flooding was partially interrupted at night), they reacted by developing systems capable of receiving and absorbing the flooding light. We may distinguish two such systems which will be represented in two pictures.

PICTURE Nº 5A

The World of Carbohydrates

One of the two systems, the chlorophyl in plants, receives the flooding waves, catches them and stores their energy to transform water and carbon dioxide into carbohydrates. Thus, essentially, the chlorophyl system absorbs the waves to manufacture food. It is a gigantic food mill which has supplied all of us living beings for millions of years. It also supplies heat to warm us up in the winter, for, in burning coal, we recuperate the heat of the sun stored in plants millions of years ago.

PICTURE Nº 5B

The World of Vision

Another system has been developed by Nature in which the waves reflected by bodies at the surface of the earth are transformed into electrical impulses which contribute to the drawing of a picture on a plate of living tissue, the retina (in a manner similar to the way electrical impulses draw pictures on the television screen). This system (the eyes in animals) transforms the waves into messages which give living beings information on the world around them. The eyes thus become one of the most precious assets in animal life.

Picture Nº 6

The World of Intelligence

The information about the world obtained by man through his eyes led him to a high level of intellectual achievements which made him the king of creation. This picture shows man mastering the universe by focusing his eyes on every creature, asking them how they are made and how they work, using magnifying lenses, telescopes and microscopes of various kinds. He has lately invented the electron microscopope; not long ago he invented photography. The twentieth century man is very busy trying to understand things. He wants to go to the moon and to Mars to understand better what goes on over there. His eyes created an insatiable appetite for knowledge. This picture therefore represents a hungry man, hungry for things of the mind.

Picture Nº 7

The World of Ophthalmology

Man became very conscious of the treasure he possesses, his eyes, and he is now applying his intellectual ability to the preservation of that treasure. Among the men hungry for knowledge, there is a group, the ophthalmologists, who focused their microscopes and adjusted their other modern scientific instruments on the eye itself. And among the ophthalmologists, those whom I am now addressing have decided to concentrate their efforts on the use of one of the latest methods of preserving vision —the use of cold in cryosurgery, cryotherapy and eye banks. I am honored to salute the men of vision appearing in this picture— the Cryoophthalmologists.

PART II

The challenge

The general purpose of cryoophthalmology being the improvement of ways of preserving sight by the use of modern techniques in law temperature, the challenge is to develop a long-range research program in which the latest advances in the cryosciences will contribute to the utmost to the realization of the intended aim.

Among the latest developments in biology, one is its passage into a molecular science, that is, into a science in which the processes are seen at the molecular level. In other words, molecular biology is a science in which biological structures and activities are seen at such a magnification that the behavior of the molecules can be visualized; and molecular cryobiology is a science in which the behavior of the molecules involved in the processes of freezing, thawing and related phenomena becomes visualizable. Now, the molecule of water measures three angstroms across (one angstrom is one ten-millionth of a millimeter), and to visualize its activities one would need a magnification of several millions (a magnification of one hundred millions would enlarge a molecule of water to the size of a tennis ball). Our task may thus be described as consisting in observing and picturing, at very high magnifications, what happens when biological materials are frozen and thawed.

In the outline of the prerequisites for the research program in cryoophthalmology that I am now to present, I shall first enumerate the essential points on which information is required; then I will illustrate the mode of approach and the results obtained in a comparable research program on the freezing of muscle, in which we have been engaged for several years in our laboratories; finally I will examine some of the practical ways of meeting the challenge.

A. The Fundamental Data Needed in the Program

The program may be described in a general way as follows; to trace in each component part of the eye the behavior of the molecules during the freezing and thawing processes, to determine the characteristic features and the distribution of the ice, and to find what changes the growth and the melting of the ice bring about in cells and tissues. The actual problems to be investigated may be classified into the following four categories:

1. *Structure of the Component Parts of the Eye:*

(a) Anatomy of the parts at the cellular and molecular level,

(b) Water content and distribution of the water in tissues and cells,

(c) Chemical composition,

(d) Physiological properties.

2. *The Freezing, Storing and Thawing Conditions*:

(a) Freezing and storing temperatures,

(b) Freezing and thawing rates and their controlling factors,

(c) Time of exposure in the frozen state.

3. *Effects of Freezing, Storage and Thawing*:

(a) Type, number, size, of ice particles, and their distribution in tissues and cells,

(b) Amount of ice formed, and amount of nonfrozen water,

(c) Degree of dehydration by freezing,

(d) Chemical effects of freezing, storing and thawing (such as denaturation of proteins),

(e) Physiological effects of freezing, storing and thawing (such as changes in permeability),

(f) Effects of cryoprotective agents.

4. *Supplemental Information of Import to the Surgeon or Physician*:

(a) Parts which must remain viable and parts replaceable by synthetic substitutes,

(b) Particular properties which must be preserved (such as permeability, turgor and transparency of the cornea),

(c) Problems related to immunity.

Note: This table is a summary of the program that I presented in a previous paper (Luyet, 1966) to which I refer the reader for more details.

B. Mode of Approach Followed in Our Cryobiological Studies on Muscle

Structure of Muscle. A muscle consists of fibers, that is, of thread-like structures of a diameter of the order of 20 to 80 micra, arranged in a more or less parallel direction (Fig. 1, D). A fiber consists of many fibrils, which are also parallel threads measuring about 1 micron in diameter. Diagram A of Fig. 1 represents, at a magnification of 50.000, a cross section through two fibrils *fl*

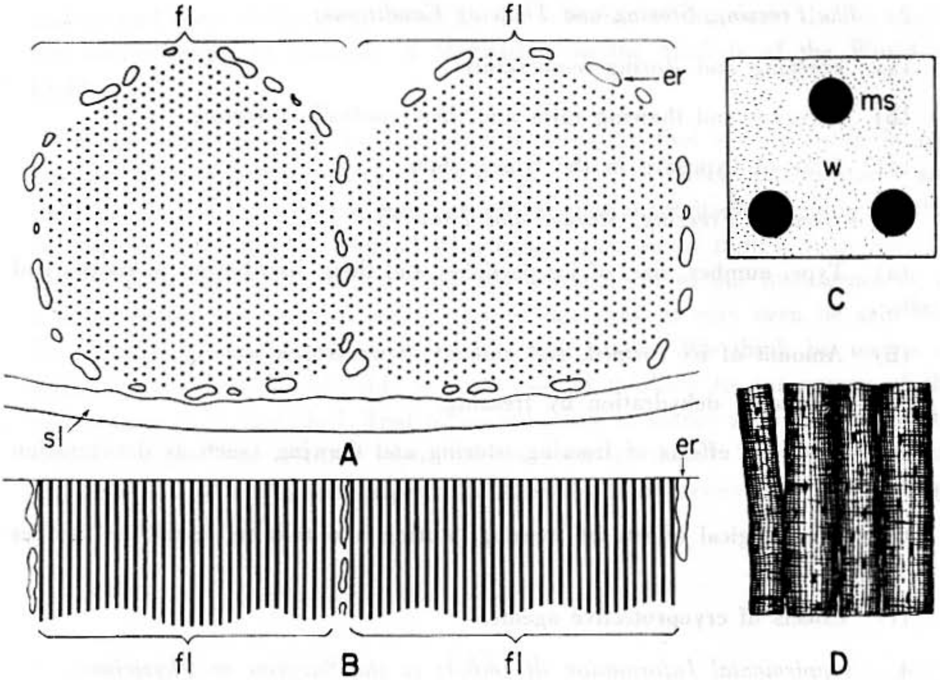

Fig. 1. Diagrams representing the structure of a muscle fiber. A. Cross section through a peripheral region, showing two fibrils (*fl*), *the endoplasmic reticulum* (*er*), and the sarcolemma (*sl*). x 50.000. B. Longitudinal section through the part of the fiber represented in A. C. Portion of A enlarged 10 times to show the relative dimensions of the myosin filaments (*ms*) and of the molecules of water (*w*). D. Group of fibers seen at a magnification 100 times lower than that of A and B. (From Luyet, 1959).

adjacent to the sarcolemma *sl* (the wall of the fiber). Each fibril is surrounded by a thin reticulum, the "endoplasmic reticulum" *er*. Diagram B represents the same structure in longitudinal section. A fibril consists itself of regularly arranged myofilaments, indicated by the fine dots in Diagram A, and, at a magnification ten times higher, by the large black spots marked *ms* in Diagram C. The myofilaments are bundles of molecules of myosin of which the diameter is of the order of 10 millimicra (100 angstroms). The space between them, about 20 millimicra, is occupied by the sarcoplasmic fluid, an aqueous medium of which the molecules of water are marked W in Diagram C. — The problem now before us is to find out what happens, at the molecular level, when the system freezes.

Freezing of Muscle. When the water in the sarcoplasmic fluid is transformed into ice, the structure may be considerably distorted. This is illustrated in a comparison of the two photographs of Figure 1. Photo. 1 is an electron micrograph of a normal, not frozen fibril showing the individual filaments and the transversal bands or lines A, I, Z and Z and M; and Photo 2, an electron micrograph of a frozen fibril showing how ice particles (now blank spaces) had grown myofilaments, pushing them aside into bundles, and to what extent the cross striations are dislocated. This case will suffice to illustrate the relationship between the dimensions of the ice particles and the structural disturbance. By reducing

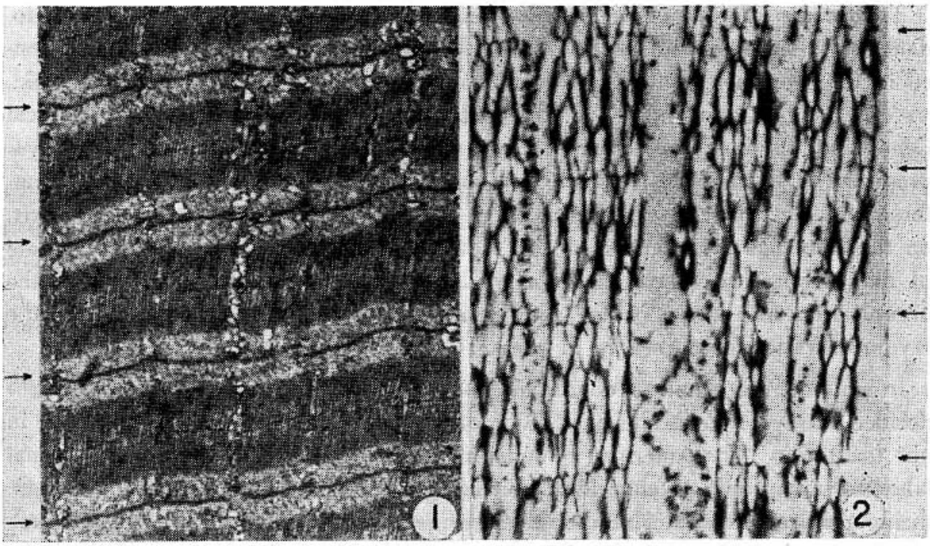

Fig. 2. Electron micrographs of longitudinal sections of frog's muscle fibers. Photo. 1: from control, not frozen fiber. Photo. 2: from a fiber frozen at -150°C. and vacuum-sublimed. The arrows on each side point to the Z lines of the muscular striations in the two specimens. In the frozen fiber, the myofilaments have been aggregated into bundles squeezed between the ice particles, now represented by blank spaces, which measure about 0.5 x 0.2. u. Magnification: 14000 x. (From unpublished files of Menz and Luyet.).

the size of the particles one can reduce, or even avoid the disturbance (see paper by Menz and Luyet, 1961). — Although I mention only mechanical effects of freezing here, one should not conclude that all injury by freezing is of mechanical origin.

C. Toward a Practical Realization of the Program

A research program covering adequately the fundamental problems in cryoophthalmology is an undertaking of major dimensions. The research itself should be preceded by a survey of the present status of our knowledge (1) on the ultrastructure of the component parts of the eye, as studied, for example, by electron microscopy, (2) on the mechanism of visual perception (sa established by physiologists and biochemists), and (3) on the structure and functions of organis of vision in lower forms of life, as investigated by embryologists, comparative anatomists and comparative physiologists (as an example of the work of the latter, I will mention the studies conducted on visual perception in flies at the California Institute of Technology).

To provide surveys of such, or of similar studies, to keep the surveys up-to-date, and also to coordinate the research conducted by the cryobiologists interested in basic problems with the research on cryopreservation in the medical field, we (Lieutenant-Commander V. Perry of the Tissue Bank of the U.S. Navy Medical Center, and I) inaugurated, three years ago, a series of conferences, the "Cryopreservation Conferences", which have been held annually since. The first conference dealt with the cryopreservation of skin and of cornea. The first part, on the skin, has been published and I refer the reader to the introductory note of that publication (Luyet and Perry, 1966) for further information on the organization and scope of the Conferences. The second part dealing with the cryopreservation of cornea is in press.

In annother attempt to coordinate basic cryobiology and cryomedicine, I decided last year to establish a "Biomedical Laboratory" as a separate unit in our Institution "The American Foundation for Biological Research". Our original laboratory continues to concentrate on basic biological and biophysical research, the biomedical laboratory studies the cryopreservation of tissues or organs, such as are stored in tissue banks. But the overall emphasis in the operation of the two laboratories (one in the Washington area, the other in Madison, Wisconsin) remains the coordination of basic and applied research.

The next and last question is: what do we do practically to meet the challenge about the coordination of basic and applied research in cryoophthalmology? I would like to take this opportunity to propose that we arrange a meeting in which specific plans for some collaborative undertakings be discussed.

This paper is a slightly modified version of the talk given by Prof. B. Luyet at the opening session of the Society for Cryoophthalmology, in Las Vegas, New Mexico, on January 9, 1967.

Wisconsin.
The American Foundation for Biologics, Research