Basic proposal to NASA, May. 1963

THE ESTIVATING LUNG FISH

It seems likely for many reasons that there exists in the world a powerful agent, hormonal in nature, which is capable of suppressing the metabolic activity of biological tissues. Thus, in a sense, it would be the opposite of the thyroid hormone, which is well known to medical science. The importance of such an agent, if it were obtainable, spreads into many fields. It would certainly be a basic factor in both the process of growth and of aging. It may relate to hibernation, with its potential for future space travel and for the survival of man subjected to intense irradiation. Its utility in the field of medicine might be extremely great. It is possible that it would be desirable to combat all the high metabolic reactions which occur as a response to stress of many types, such as severe injury, burns, infections, and the like. It may play an important role in the prophylaxis of the arteriosclerotic process (the core of the aging process); it might find immediate important practical application in the increasing development of heart surgery and in the transplantation of organs and tissues. Obviously, the agent, if broadly applicable biologically, would have immense potential.

Interest for a number of years has been centered in the attempt to analyze the secret of the true hibernating mammal, such as the golden hamster or the ground hog. The basic mechanism of hibernation is as yet unsolved, but it seems possible that the marked lowering of the metabolic rate (oxygen consumption) of the hibernator when he is cold may not be merely due to the lowering of his temperature. On the contrary, it may be due to a basic change in his metabolic rate which, in the presence of a low ambient temperature, reduces his heat production and thus allows his body temperature to fall. The ground hog, hibernating with a body temperature of 4° C for several months is an extraordinary biological situation.

Perhaps even more extraordinary is the ability of a group of animals in the world who have the capacity to estivate; that is to say, to enter a torpid state in the heat of summer. It would appear that these animals have the capacity to reduce their metabolism in an ambient environment of high temperature. Clearly, their metabolic reduction is not due to diminution in temperature, although what is their temperature in the estivating state is, I believe, unknown; in fact, not much concerning the body temperatures of estivators is known.

Prominent in the zoological world of estivators is the lung fish of Africa. This extraordinary throw-back to one of the very earliest forms of fish has a gill system incapable of supporting life under water thus requiring him to surface to utilize his lungs for respiration. He has shown an almost incredible degree of adaptivity to a markedly changing environment.

His distribution is world wide and he is commonly found in Australia, South America and South Africa. The lung fish is of the group _Dipneui_, of the family _Lepidosirenidae_. The _Lepidosiren_ inhabit South American and rarely estivate. The _Protopterus_, however, living in the very cyclic weather of Central Africa, have developed estivation as a primary means of survival. Of the four recognized species, _dolloi_, _aethiopicus_, and _annectens_ are the best known. The most powerful estivator of the entire group is the _Protopterus annectens_ of Africa, who is an obligate cyclic estivator. However, Protopterus aethiopicus is also a potential estivator, and does so whenever the environment becomes dried out. This depends in the vageries of cyclic "wet" and "dry" year.

This remarkable animal, which is found in the central portion of Africa, normally lives in marshes which become wet and swampy during the rainy season (March to October), but which dry out to hard baked mud during the dry season of the year (November to February). The Protopterus which is thought to be a fish which as undergone little change for over 70 million years, solved this problem as follows: During the swampy period of the year the fish swims in the murky, muddy waters, glides along the muddy surface like an eel and is omniverous, although most of his diet is apparently vegetable matter. He burrows in the mud and the female lays eggs guarded by the male until the larvae appear. As the rains cease and the drought begins, the swamps begin to dry. While the mud is still slimy, the Protopterus digs a burrow six to ten inches wide almost directly vertically into the mud by swallowing the mud and ejecting it through his gill crevices. In the bottom he rounds himself an elliptical den and one day he coils himself into a coil with his tail covering his head. His body then begins to excrete a slimy substance which hardens as the mud dries until it is a firm cocoon. Interestingly, his head is always in the vertical position, facing up the tunnel, and a channel forms on the inside of his mouth leading to the entrance to his lung. Thus, in the estivating state, the animal is respiring slowly through this one opening in the cocoon. He remains in this condition motionless and in a state of torpor for three to four months. Urine formation is suppressed, and high internal levels of urea accrue. When the rains come again the cocoon is dissolved, the fish re-animates and the cycle in the now muddy and watery swamp begins again. It is thought that the mechanism of re-animation is the hypoxia of being covered by water and thus being unable to breathe.

The metabolism of the estivating aethiopicus was studied by Smith, and observations were made on changes in the body composition, on the pattern of excretion on the oxygen consumption, and the effect of temperature and of thyroxine on metabolism. The oxygen consumption fell gradually to more or less 15% of that in the active state. In a fish estivating for 18 months, the O_2 consumption was 8c.c./Kg/hr. at compared to 20 c.c. normally. When the temperature was raised from 20° C to 30° C in an experimentally encysted lung fish, the O_2 consumption rose from 7.8 c.c./Kg/hr. to 27 c.c.. However, the animal did not awaken. After the administration of Thyroxin, the metabolic rate rose sharply, reaching a peak in 4 to 5 days, then falling to pre-injection levels by the 10th to 12th day. Again the fish did not arouse. These last results have been confirmed by other investigators.

The fish tolerates incredibly long periods of estivation before death ensues. The two fish allowed to progress to final inanition, the fasting periods were 629 and 473 days respectively.

It is stated that as the dry season approaches, the animal builds up increasing amounts of a fatty tissue which surrounds the kidneys and the gonads along the posterior portion of his body. During the period of estivation it is said by some that the fat gradually disappears and it was considered by them to be a source of energy. It is also said by others that the fat does not disappear and that the fish metabolizes dowly his own muscular tissue. In either event, it is my strong suspicion that, like the brown fat of the mediastinum of the ground hog in Canada, this fat represents the estivating gland of the Protopterus and his secret lies in a hormone elaborated here and released slowly from it.

It is suggested that an intense biologic and biochemical investigation of this material might lead to the demonstration, identification, and the subsequent synthesis of a potent antimetabolic endocrine agent.

Since it is possible to force the <u>P. aetiopicus</u> to undergo experimental encystment and enter "estivation" by the simple process of allowing his environment to become dried out (irrespective of the time of year), this animal provides the ideal subject for laboratory investigation. His habitat is the high plains area of the upper Nile basin in Sudan, Uganda, Kenya, Republic of the Congo, and Tanganyika. He abounds in the eastern estuaries of Lake Victoria, where he can be easily harvested by netting when in the active state, or by patient diggings when in the estivating state.

It is proposed, therefore, that an expedition be formed to go to Kenya during the month of January, 1964, with initial headquarters in Nairobi. After recruitment of pertinent and informed local personnel (members of the Natural History Museum, native helpers and fishermen, etc.) the field expedition would move to Kisumu, Kenya, or to Jinja or Kampola, Uganda, to undertake field studies and to collect specimens of <u>Protopterus ethiopicus</u> to bring back to the United States for further extensive studies.

If the year has been a dry one, large fish (20 to 50 lbs.) in the natural estivating state may be obtained by finding the mouth of their holes and digging them out, leaving the cocoon with its surrounding mud pack intact. O_2 consumption studies of the fish could be attempted by capping the mouth of the tunnel, for correlation with subsequent analysis. This has never been done. In addition, smaller active fish (1/2 to 3 lbs.) can be caught in the estuaries of the lake and preserved in muddy water. The fish are extremely durable (in the experience of Smith) and will tolerate the trip to the United States easily in 2 and 5 gallon cans half filled with muddy water.

It is hoped that at least 20 large estivating fish and about 150 smaller active fish could be procured. With proper management, at least 90% should survive their journey west, which could best be achieved, I think, in a military plane, preferably a jet. The environment must be temperature controlled, of course. The period required for this expedition should be approximatly six weeks. The salaried personnel from the U.S.A. would consist of:

a. Dr. Henry Swan, Director
b. Ph.D., Staff physiologist
c. Ph. D., Staff Biochemist
d. Two biochemical technicians
e. Two physiological technicians

The consulting personnel would consist of:

a. Dr. Wilfred Bigelow, Toronto, Canada (on leave from the University of Toronto)
b. Curator of the Royal Museum of Natural History, Nairobi
c. Commander Morsmakers, (of the Royal Marines), (local expert in the finding and excavation of estivating <u>aethiopicus</u>)

Upon returning to the United States, a portion of the fish would be sent to each of three research centers:

A. Colorado State University (Dr. Swan)
B. Toronto University (Dr. Bigelow)
C. Duke University (Dr. Brown)

Here, vigorous study of the physiology of estivation and the careful search for an active antimetabolic agent would be pursued, with free and constant interchange of information. The direction of further studies would be determined from the initial experience.

The promise or falsity of the basic premise should be established within six months, i.e. by July 1, 1964.

BIBLIOGRAPHY ON LUNG FISH

General References:

Dean, Bashford — A Bibliography of Fishes (3 Vols.) American Museum of Natural History, N.Y.

Boulenger, George A. — Catalogue of the Fresh-Water Fishes of Africa in the Britsh Museum (4 Vols.) London, 1909-16.

Poll, Max. — Les genres des poissons d'eau doce de l'Afrique. Royaume de Belgique, Minister des Colonies, Brussels, 1957

Smith, Homer W. — From Fish to Philosopher, Little Brown and Co., N.Y., 1953.

Herold, Earl S. — Living Fishes of the World, Doubleday, N.Y. 1961

Jordan, David — Fishes, D. Appleton Co., 1925

Fishes of the World, The Country Man Press, Woodstock, Vt.. 1962

Hildebrand, Samuel — The Smithsonian Series, Volume 8, Fishes, Amphibians, and Reptiles, Part I.

Specific References:

Leloup, Jacques — Influence de "l'estivation" sur le fonctionnement Thyroidien du Protoptere - C.R. Acad. Sc., Paris, 246,830, 1958.

Bouillon, Jean — The Lung Fish of Africa, Natural History 70 (2); 62, 1961.

Greenwood, P.H. — Reproduction in the East African Lung Fish, Protopterus aethiopicus, 1958 (Ref. unknown)

Mohson, T., and Godet, R. — Action of Thyroxine on the Rate of Oxygen Consumption of the Lung Fish (Protopterus) Nature 185 (4706); 180, 1960.

Parker, W. — On the Anatomy and Physiology of Protopterus annectens. Trans. Royal Irish Acad., 30; 109, 1892

Smith, Homer W. — Metabolism of the Lung Fish, Protopterus aethiopicus. J. Biol. Chem. 88;97, 1930

Smith, Homer W. — The Metabolism of the Lung Fish. I. General Considerations of the Fasting Metabolism in Active Fish, J. Cell. Comp. Physiol. 6; 43, 1935

Specific References Contd.:

Johnels, A. and Svensonn, G.	On the Biology of Protopterus annectens Arkiv for Zoologi, 7; ?, 1954.
Cury-Lindahl, K.	On the Ecology, Feeding Behavior, and Territonality of the African Lung Fish Protopterus oethiopicus Arkiv for Zool. 9; 479, 1956
Morlier, G.	L'enkystement du Protopterus aethiopicus (Heckel) Ann. Soc. Roy. Zool. Belg. 87; 211, 1957
Blanc, ., D'Aubenton, .,et Plessis, Y.	Etude de l'enkystement de Protopterus annectens Bull. I.F.A.N. 18; 843, 1956
Brien, P., Poll, M., and Bouillon, J.	Ethologie de la Reproduction de Protopterus dolloi, Ann. Musee Roy. Congo, Belg. 71; 3, 1959