NAVAL MEDICAL RESEARCH INSTITUTE
NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND - 20014

6 pages
Code-1
CR-76771
N66 32441

NMRI-022-jo
3900
Ser 1726

14 JUN 1966

HARDCOPY(HC) $1.00
microfiche(mf).50

Dr. George Jacobs
Chief, Physical Biology
National Aeronautics and Space Administration
400 Maryland Avenue, S.W.
Washington, D. C.

Dear Doctor Jacobs:

This letter will confirm our telephone conversation in which I indicated that Contract R-63, Freezing and Drying of Living Cells, will be financed internally in the future and that it will not be necessary for us to request renewal following the termination of the present contract on June 30, 1966. I enclose a copy of a memorandum from the Head of the Tissue Bank which will amplify the circumstances leading to this decision.

During the four years in which we have received financial assistance from NASA, our rate of progress has been uneven and we have explored some blind alleys, particularly in relation to the initial study of freezing and drying resistance in the nematode which terminated unproductively with the departure of Dr. Burns. However, I am pleased to report that the ultimate outcome has been all that I could have wished. The design and construction of continuously recording calorimetric apparatus, completed under our NASA contract, enabled us to demonstrate a consistent relationship between dehydration and freezing injury and to rule out previous theories regarding the mechanical effects of ice crystals which have dominated cryobiology for many years. Based largely on these calorimetric measurements, we have been able to assemble a hypothesis for the mechanism of freezing injury and its prevention by cryoprotective agents, and we can now explain the influence of various freezing rates and cryoprotective agents on the basis of their effects on cell dehydration.

This hypothesis has led us to a series of experiments which have demonstrated that, contrary to previous assumptions, a substantial proportion of the freezing injury seen with low concentrations of cryoprotective agent can be prevented by very slow freezing and thawing. With our better understanding of the mechanism of freezing injury we have identified two new approaches to low temperature preservation. The first of these is the use of low concentrations of cryoprotective agents which would be inadequately protective at very low temperature but which afford good storage at moderately low temperatures of the order of -20° C. There appear to be applications for this compromise approach in materials which are injured by the high concentration of

cryoprotective agents usually necessary to achieve very low temperature preservation.

Since preliminary supercooling and the lowering of the supercooling temperature through solutes has been a vital element in our hypothesis of injury, we have been led to further investigation of supercooling and its control. We find that supercooling to temperatures of the order of -10° C can be easily achieved and indefinitely maintained using a variety of solutes which do not have the toxicity of the cryoprotective agents and, in many situations, do not penetrate the cell. Even these modest reductions in temperature have been found to confer a substantial increase in the duration of storage which can be achieved. We are exploring the application of these two preservation techniques to leukocytes and platelets and to certain of the protozoa which are of practical importance but for which no satisfactory preservation method has yet been devised. I am preparing an extensive report of our progress for the director of research grants and I will see that you also receive a copy.

There is no question but that these theoretical and practical developments have been to a great extent made possible by the support that we have received from NASA. I particularly appreciate the fact that you have not hounded us for a continual flow of tangible results but have had sufficient confidence to permit our investigations to progress free of pressure. I feel that the developments summarized above will have more than justified your confidence.

Very sincerely yours,

HAROLD T. MERYMAN, M.D.
Tissue Preservation Research Division

Encl:
(1) Copy of Memo