TRANSPORT OF LIFE IN THE FROZEN OR DRIED STATE*

BY

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We received with keen appreciation the invitation to contribute a paper on space transport of life in the frozen state to the Space Medicine Symposium, but we accepted with diffidence. We felt we had some knowledge of the biological effects of freezing, but we hesitated to stick our neck out into space. We did what could be done in a few days by consuming a heavy meal of space literature, much of it written by distinguished members of the British Interplanetary Society. Even so, our contribution relates to biology rather than to medicine, and to the planet Earth rather than to space. Moreover, it deals only with the freezing and drying of living organisms. The justification for the inclusion of the dried state is, firstly, that suspended animation is achieved in nature by desiccation more often than by freezing, and, secondly, that the ultimate aim of all biologists engaged in the preservation of life at low temperatures is to graduate from freezing to freeze-drying, so that the material can be kept on the shelf rather than in the deep freeze. We should add that even in our own field we shall make statements to which there are numerous exceptions.

Hazards Awaiting Space Travellers

We have confined our recent literary researches to the solar system; we read that on the moon there is no atmosphere, little if any water, and wide and sudden swings of temperature; that on Venus an atmosphere of CO₂, lacking water vapour and oxygen, lies above thick clouds which may be dust, petroleum products, or something else; that on Mars the temperature is comparatively benign, but that there is little water, except when the ice caps melt, little or no oxygen, but a great deal of CO₂ in its tenuous atmosphere; that on Mercury the temperature difference between the shady and sunny sides may be as much as 650° C., though the twilight zone, where something analogous to night and day occurs because of the oscillation of the planet, might be more tolerable from the thermal point of view. We read that Jupiter, Saturn, Neptune, and other outer planets have bitterly cold atmospheres composed of methane, ammonia, and hydrogen. To these hazards which await space travellers on arrival at any of the other planets must be added the hazards of getting there. Among these may be mentioned cosmic rays, especially those trapped immediately above the earth's atmosphere, hard x rays given out by the sun, particularly at the time of sunspot activity, and intense ultra-violet irradiation. Extremes of temperature will also be encountered. This catalogue may well be considered formidable by the Earthly biologist, and the object of this paper is to see if there is any relevant information in our accumulated knowledge of the effects of freezing and drying on living organisms.

Properties of the Lower Organisms

Life, in the sense of matter showing growth, reproduction, and irritability, as we know it on Earth, depends essentially on a cycle involving water, oxygen,

carbon dioxide, and fixed nitrogen, and a temperature at which their interaction is possible. In lower organisms the chain of events is often arrested temporarily by drying or cooling without permanent damage to the system. Moreover, many lower organisms and most plants produce spores or seeds which have a very low water content and a negligible respiration rate, and thus have a high resistance to cooling, drying, and lack of oxygen.

Two hundred and fifty years ago Leeuwenhoek found that the little animals which he had observed microscopically in drops of water from ponds and gutters survived complete desiccation. In the later eighteenth century Spallanzani found that a few of the similar animals in hay tea survived freezing at the lowest temperature which he could produce, which was about -20° C. More recently, Russian scientists were able to culture bacteria and protozoa from frozen soil which they obtained in Siberia when digging up carcasses of mammoths frozen at least 50,000 years ago in the Pleistocene Age. Becquerel found that algae and fragments of lichens, spores of bacteria and moulds, and also minute animals including Tardigrades and Rotifers, survived the combined effects of dehydration, high vacuum, and cooling to temperatures approaching absolute zero, -273° C. He calculated that organisms which would survive only one year at +10° C. could remain in suspended animation for billions of years under these conditions. Arrhenius thought that, in the dry state, living forms had already been wafted through outer space and that the earliest forms of life on our planet might have come from some distant star.

To return to Earth, there are well-authenticated examples of seeds of higher plants which have remained viable for hundreds or even thousands of years under highly adverse natural conditions. Professor Keilin, in a recent lecture, cited the case of a water-lily which had become extinct and was known only from the work of early Chinese artists. Then, in about 1932, seeds of this lily were found in a desiccated and encrusted state in the bed of a lake which, according to local records, had been dried up for a thousand years. Some of the seeds were sent to Kew Gardens, and a few, after being cleaned up with sulphuric acid, germinated and the once extinct lily bloomed again. For the sceptical, we should add that it has been possible to check the antiquity of the remaining lily seeds by radioactive carbon dating. On the other hand, according to Brimble, and contrary to popular belief, wheat grains found in the tomb of Tutankhamen were not viable.

Preservation by Freeze-drying

Modern techniques of freeze-drying will no doubt extend greatly the types of life that could be preserved for long periods in the dried state, though, from the point of view of the biologist, it is unfortunate that work on reviving organisms after prolonged periods of suspended animation has lately been concentrated on preserving pathogens. So far as the biologist is concerned, therefore, it is likely that lower animals as well as spores and seeds of plants could be landed in

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good condition on another planet, provided that the effects of irradiation and overheating could be avoided. In fact, it would be difficult or impossible to exclude them from space vehicles, particularly if higher animals were transported. Unless the most stringent precautions were taken they would inevitably go along as fellow travellers. Further, some of the photosynthesizing organisms which take in an excess of CO_2 and put out an excess of oxygen might even become established on Mars. The chances would be particularly good in the case of some of the xerophytic plants, especially the symbiotic lichens, which to some extent provide their own micro-climate. It has, in fact, been pointed out that the apparent vegetation on Mars, if similar to anything found on Earth, has possibly evolved along the lines of the lichens. The possibility of carrying out a colossal experiment in evolution by the colonization of Mars with a few selected species of lower organisms is particularly fascinating in this year of the Darwin Centenary.

The problem of establishing animals on other planets is very different. Lichen-like vegetation might supply the basic plant food, but, even on Mars, the scarcity of oxygen, and low atmospheric pressure, must mean that the establishment of Earthly animal life, especially in its more complex forms (for example, mammals), would require the provision of micro-climates in which oxygen tension, humidity, and probably also temperature were adjusted. If animal life, in the sense of self-propagating matter ultimately dependent on plant food, has evolved at all on the planets, it must have done so along lines unknown on Earth. This may well have happened. It is not impossible to imagine matter based on ammonia, methane, and silicon instead of on water, oxygen, and carbon, having the properties of growth, reproduction, and irritability, and therefore showing the classic criteria of life. At the temperatures prevailing, however, any life based on biochemical reactions will be pretty sluggish, particularly on the outer planets, and it is to be doubted if evolution can have proceeded far. But, fortunately, these problems are outside our scope.

A Biological Problem

The biological problem of transporting any of the higher forms of animal life to the planets is sufficiently formidable. Clearly, a state of suspended animation (anabiosis) in which food, water, and oxygen requirements were abolished, would be highly advantageous in transit, and here we can be guided by a rapidly expanding body of knowledge on the long-term preservation at low temperatures of animal organisms and of their essential germ cells. Alaskan and Siberian insects provide good examples of anabiosis induced in nature by cold and persisting for many months. There are now reports of moth larvae remaining viable after being frozen and stored in liquid air under experimental conditions. Again, intertidal molluscs, necessarily exposed to air temperatures at low tide, are in some regions frozen and thawed twice a day during the winter. These molluscs are remarkably tolerant of having a large part of their body water converted into ice and of the hypertonicity of the residual fluids which results therefrom.

The situation is very different with vertebrate animals. Even the cold-blooded ones, which can adjust their bodies to a wide range of temperatures above zero, do not tolerate extensive freezing. There is no evidence that vertebrates can survive the conversion of all their body water to ice under natural conditions. Stories

of revival of fish and frogs found frozen solid in blocks of ice probably depend upon the lower freezing point of blood and tissue fluids, so that the animals could remain unfrozen though surrounded by ice and would survive until they died of oxygen lack.

The young or hibernating warm-blooded animal has something in common with the cold-blooded animal in that its body temperature can be reduced to a few degrees above 0°C . without damage. The hibernating bat can actually survive in the supercooled state at several degrees below zero provided that it does not freeze. In all these cases, however, heart beat and respiration still proceed slowly, so that the state is not one of suspended animation. There is no evidence that the cold-blooded vertebrate or the young or hibernating warm-blooded animal has any dispensation from the damage caused by conversion of body water to ice. In adult non-hibernating mammals the position is still more difficult because breathing and heart beats cease when the internal body temperature is reduced to a level somewhere between 25° and 15°C . When cooled below that range the mammal is in a dangerous state without respiration or circulation, and, until recently, death was, in fact, supposed to be inevitable. This is now known not to be so, and by special methods mice, rats, and dogs and monkeys have all been revived from deep body temperatures approaching 0°C . after cessation of breathing and heart beats for up to one hour. Recovery is usually complete and, remarkably, tests on resuscitated rats have shown no impairment of memory or learning ability following the severe anoxia. The dogs and monkeys have shown no change in reflexes or in behaviour.

Supercooling

In the case of the hamster, a potential hibernator, which can be handled more easily in the range of body temperatures above freezing-point, the experiment has been pushed further and body temperature taken below zero by immersion in a bath at -5°C . In these circumstances one or other of two things happens: the animal may start to freeze, or it may supercool. In the freezing animal, body water begins to be converted to ice in the superficial tissues, in which case the deep body temperature drops only very slowly below the freezing-point of the blood, -0.6°C . Such animals have been resuscitated undamaged after being below freezing-point for up to one hour, at which point some 50% of the body water had been converted to ice. During supercooling the body temperature is not maintained by the latent heat of crystallization of water, and falls rapidly to that of the surrounding bath. The supercooled state may be terminated by the sudden crystallization of ice throughout the tissue fluids, which results in a rapid rise of body temperature to freezing-point and is very dangerous to the animal. If spontaneous crystallization does not occur, supercooled animals can be revived in perfect condition.

Kalabukhov recently suggested that bats and other mammals might be supercooled to lower temperatures and so kept in a comparatively stable state. It may be that the first space explorers will have bats in the spaceship. It must be emphasized, however, that the partly frozen or slightly supercooled animal without heart beats and respiration is in a highly unstable condition because many of the physico-chemical processes of life are going on at a reduced rate and almost certainly at relative rates quite different from normal. Chaos therefore arises. The same applies with even greater force to bodies without heart beat or

respiration at temperatures between 0° C. and +15° C., in which biochemical disorder is running wild. Much lower temperatures will be required to arrest biochemical and physical changes and so obtain a stable state in which the animal can be preserved indefinitely. The biologist is thus not within sight of obtaining long-term suspended animation in warm-blooded animals, or any other vertebrate, but it would be a mistake to suppose that he never will succeed.

In man, body temperature is now commonly reduced to about +28° C. for surgical purposes, but at this temperature the heart is still beating slowly, and natural breathing can, if necessary, be supplemented by artificial respiration. And here we would like to protest against the use of the expression "deep freeze" to describe hypothermia induced for surgical purposes. The condition is not one of deep freeze, and the implication is sadly misleading. The techniques actually used in human patients are remarkable enough without exaggeration. We refer, for instance, to Melrose's method of elective cardiac arrest induced by injecting potassium ions into the coronary circulation of subjects in which a systemic circulation of oxygenated blood is maintained by an artificial heart-lung machine. Only recently, Niazi and Lewis reported that a patient had survived cooling to a deep body temperature of +9° C. without circulating her blood stream through a pump oxygenator. The heart was at a standstill for one hour. After re-warming, the patient recovered consciousness and survived 38 weeks thereafter without any apparent alteration in neurological or intellectual functions. Before treatment she had been in the terminal stages of inoperable pelvic carcinoma with abdominal and pulmonary metastases. She eventually died from spread of the original disease. Nine degrees above freezing-point is the lowest body temperature so far recorded from an adult human being who subsequently recovered. In the future, temporary cooling to this degree may prove of great value in medicine and surgery and become a routine practice.

Conclusion

Science fiction notwithstanding, the prospect of suspending animation for indefinite periods in man by freezing him is remote. When it becomes practicable, will it help the exploration of space? Possibly: to carry a proportion of the crew of a spaceship in a state of suspended animation would reduce oxygen and food requirements. But that is not all. A frozen body would presumably be partly or even wholly immune from the effects of violent acceleration or deceleration, provided that it was well supported to prevent breakage. Again, the effects of whole body irradiation are greatly reduced at low temperatures. The dose of whole body x-irradiation which is lethal to 50% of mice is about 500 r. When their body temperature is reduced to 0.5° C. and heart beat and respiration have ceased, the corresponding figure is about 1,800 r—a greater degree of protection than is afforded by any of the known protective substances. The effect is probably associated with the state of comparative anoxia of the cold animal, but that degree of anoxia could not otherwise be obtained without severe damage. How far the protective effect of cold at 0.5° C. will be intensified at lower temperatures is not known. This aspect of the subject is worth pursuing.

We must also bear in mind that suspended animation for space travellers would avoid the effects of old age,

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To-day's Drugs

With the help of expert contributors we publish below notes on a selection of drugs in current use.

Palfium (M.C.P. Pure Drugs).—This is a new, very powerful analgesic, discovered in Belgium, which has received some publicity under the names "R.875" and "jetrium." It is a synthetic drug of a new chemical type; its formula is (+)-1-(β-methyl-γ-morpholino-αα-diphenyl-butyl)pyrrolidine and its official name is dextromoramide. Tablets containing 5 mg. of the base in the form of its bitartrate, and ampoules containing 5 and 10 mg., are supplied.

This drug is at least as potent as morphine, and equally dangerous in its liability to cause addiction. The recommended dose for relieving post-operative pain is 5 mg. every six hours. The manufacturers say that single doses of 20 mg. may be given, and up to 80 mg. or even more in 24 hours, for inoperable carcinoma. However, a high incidence of side-effects has been reported on a dosage of 10 mg. every six hours; these include giddiness, which was frequent, severe respiratory depression, nausea, sweating, and headache.

A report of toxic side-effects, with references to the literature, has recently appeared.¹

N.H.S. basic price: 100 tabs. 5 mg., 19s.; 10 amps. 5 mg., 5s. 9½d.; 10 mg., 7s. 0½d.

REFERENCE

- ¹ Brisset, C., and Dereux, J. R., *Presse méd.*, 1959, **67**, 546.

Tympalgin Ear Drops (Siltan).—These contain phenylmercuric nitrate 0.1 g., benzocaine 1.5 g., ephedrine 1 g., phenazone 5 g., chlorbutol 1 g., potassium hydroxyquinoline sulphate 0.1 g., propylene glycol to 100 ml. They are intended for the treatment of otitis media and otitis externa.

Of the seven ingredients, two, phenylmercuric nitrate and potassium hydroxyquinoline sulphate, have an inhibitory action on the growth of bacteria and fungi. Three ingredients, benzocaine, phenazone, and chlorbutol have an analgesic or sedative action on mucosal surfaces (chlorbutol is also a fungistatic), while ephedrine, by its decongestive action, facilitates drainage of exudate from the middle ear.

While it is logical to combine substances with an antibacterial, decongestive, and sedative action, the advantages of using no fewer than six ingredients (apart from the vehicle) are not obvious. Benzocaine and ephedrine do reduce pain and congestion in some cases. A point to bear in mind is that it is doubtful whether fungi are ever pathogenic in otitis media or externa; it is probable that they are present only as secondary and harmless contaminants. The topical application of cortisone derivatives is of special value in otitis externa.

N.H.S. basic price: ½ fl. oz., 2s. 7d.

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from which they might otherwise begin to suffer on the longer journeys, and dispose of what we understand is the very tricky problem of burial in space. Finally, we would like to say this: it is evident that the first problems of space travel lie with the physicist, the engineer and the medical physiologist. But, at a later stage, perhaps a not very much later stage, the biologist will play an important part, and he (or she) will have a fascinating time in doing so.

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