- Culpin, M. (1935). "Neurasthenia in the Tropics," *Practitioner*, **135**, 146.
- Fleischer, W. L., Stacey, A. E., Houghton, F. C., and Ferderber, M.B. "Air Conditioning Industry," *A.S.H.V.E. Jour. Sect.* (No date.)
- Huntington, E. (1924). *Civilization and Climate*, 3rd ed., New Haven.
- Johnson, J. (1815). *Influence of Tropical Climates, more Especially the Climate of India, on European Constitutions*, London.
- Kuno, Y. (1934). *Physiology of Human Perspiration*, London.
- Lamprell, B. A. (1941). "Discussion on Tropical Neurasthenia," *Proc. Ann. Gen. Meeting Assam and N. Bengal Branch of Brit. Med. Ass.*, Calcutta, p. 25.
- Levick, G. Murray (1945). "Physical Preparation of Commandos," *Proc. roy. Soc. Med.*, **38**, 97.
- MacKinnon, M. (1923). "Education in its Relation to the Physical and Mental Development of European Children of School Age in Kenya," *J. trop. Med. Hyg.*, **26**, 136.
- Markham, S. F. (1942). *Climate and the Energy of Nations*, Oxford.
- Orenstein, A. J., and Ireland, H. J. (1922). "Experimental Observations upon the Relations between Atmospheric Conditions and the Production of Fatigue in Mine Labourers," *J. indust. Hyg.*, **4**, 30, 70.
- Petersen, W. F. (1935). *The Patient and the Weather*, vol. 1, pts. 1 and 2; vol. 4, pt. 1, Ann Arbor.
- Robinson, S., Turrell, E. S., Belding, H. S., and Horvath, S. M. (1943). "Rapid Acclimatization to Work in Hot Climates," *Amer. J. Physiol.*, **140**, 168.
- Sundstroem, E. S. (1924). "Some Studies in Tropical Acclimatization," *Comm. Austr. Dept. Health. Serv. Publ. (Trop. Div.)*, No. 6.
- Vernon, H. M. (1928). "Industrial Fatigue in Relation to Atmospheric Conditions," *Physiol. Rev.*, **8**, 130.
- Weiner, J. S. (1936). "Report on Heat Stroke, Heat Collapse, etc.," Central Mining-Rand Mines Group, Health Dept. Report, Annexure 2, p. 74.
- Woodruff, C. E. (1905). *Effects of Tropical Light on White Men*, New York and London.
- Wyatt. Quoted by Vernon (1928).
- Yaglou, C. P. (1937). "Abnormal Air Conditions in Industry: Their Effects on Workers and Methods of Control," *Symp., Environment and its Effect upon Man.*, p. 82, Harv. Sch. Publ. Health, Boston.

PRESERVATION OF HUMAN SPERMATOZOA AT LOW TEMPERATURES

BY

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Several experiments have been carried out, in the last few years, on the preservation of spermatozoa at low temperatures. This work is clearly in its early stages, but already very significant observations have been made on human as well as on avian and amphibian material. Luyet and Hodapp (1938) reported that a large percentage of frog spermatozoa showed motility after being partially dehydrated in 2 M-sucrose, spread in a thin film on a mica slip, immersed in liquid air, and thawed rapidly by plunging into medium warmed to 20° C. The technique of these authors was similar to one which had been successful with plant tissues, and was based on the assumption that the essential condition for survival of the spermatozoa was instantaneous freezing, to secure vitrification rather than crystallization of the water, and instantaneous thawing. Partial dehydration, by the addition of laevulose, was also used by Shaffner, Henderson, and Card (1941), who found that about one-third of fowl spermatozoa could be revived after freezing at -79° C. Duration of freezing, up to 14 months, was not an important factor in survival rate. Spermatozoa revived after one hour at -79° C. were capable of effecting fertilization, but there was very early embryonic death (Shaffner, 1942).

In view of these results the discrepant experiments recorded in the literature dealing with human spermatozoa are of great interest. As long ago as 1897 Davenport recorded that human spermatozoa would survive freezing at -17° C. This interesting observation received little attention for many years, but in 1938 Jahnel found that a proportion of human spermatozoa could be revived after freezing in glass tubes for 40 days at -79° C., or for shorter periods at -196° C. and -269° C. Jahnel does not state the diameter of the tubes used, but he did not apparently take any special precautions to ensure the utmost rapidity of freezing and thawing. Shettles (1940) froze human semen in glass capillary tubes 0.2 mm. in diameter, and obtained a low percentage revival of the spermatozoa from -79° C., -196° C., and -269° C. A few spermatozoa revived after 70 days at -79° C. Shettles emphasizes the importance of the freshness of the specimen. Hoagland and Pincus (1942), following up the premises of Luyet and Hodapp and using liquid nitrogen, tried an elaborate series of devices to facilitate the instantaneous vitrification and thawing of human semen. However, except in one or two isolated experiments, the revival rate was very poor. No spermatozoa could be revived after vitrification in fine capillary tubes.

The results of Hoagland and Pincus are in strange contrast to those of Jahnel, whose work is not cited by the American authors, and the discrepancy shows that other factors than mere rate of vitrification and thawing have to be considered. From a practical point of view, the question is of some importance. Jahnel's results suggested that the prolonged storage of human semen at low temperatures might be possible; the results of Hoagland and Pincus, on the other hand, offered no such prospect. The experiments described below were therefore carried out on the low-temperature preservation of human spermatozoa.

Technique

Semen was obtained from several different donors; all specimens were rated normal by the usual seminological examination. No marked difference in resistance to freezing was noted between the different samples. The semen was allowed to liquefy before being used, and was not diluted. In experiments with capillary tubes the semen was allowed to rise in the tube and the end was then sealed. Unless otherwise stated, immersion in the freezing medium was always for one minute, thawing being done by transferring the film, tube, or ampoule as rapidly as possible to medium or water at 37° C. Freezing fluids, other than liquid nitrogen (-195.8° C.), were made by cooling ethyl alcohol with solid CO₂. Quart-size vacuum flasks were used as containers.

Results

In a preliminary experiment a comparison was made by immersing semen in liquid nitrogen (a) as a thin film in a platinum loop, (b) in a very fine capillary tube (internal diameter approximately 0.15 mm.), (c) in a coarse capillary tube (internal diameter approximately 0.5 mm.), (d) in thin glass tubing (internal diameter approximately 1 mm.). After thawing, no motile spermatozoa were seen in the semen from (a) or (b); they were abundant in that from (c) and (d). A repeat experiment gave similar results. It seemed, therefore, that the results both of Jahnel and of Hoagland and Pincus were reproducible, and considerable doubt was cast on the idea that speed of freezing and thawing was the primary factor in the survival of human spermatozoa. A more extended experiment was carried out in which the temperature of freezing as well as the bore of the container was varied. The results are given in the accompanying Table. Although survival at -20° C. was not

Table showing Revival of Human Spermatozoa After Exposure to Low Temperature for 1 Minute or 5 Hours

Temperature	Freezing for 1 Minute in Capillary Tubes of Diameter of			Freezing for 5 Hours in Capillary Tube of Diameter of 1 mm.
	0.15 mm.	0.5 mm.	1 mm.	
- 20° C.	0	+	++	0
- 79° C.	0	+	++	++
- 196° C.	0	++	++	++

0 = No revival. + = Some spermatozoa revived. ++ = Abundant spermatozoa revived.

prolonged, these results give little support to the view of Luyet that freezing under conditions leading to the formation of ice crystals is incompatible with the survival of the spermatozoa, but they confirm the previous result that some inimical factor is associated with freezing in a very fine capillary tube. The nature of this factor is not clear, though a surface effect of some kind seems to be indicated. Pressure as such is not likely to be involved, since Marsland and Rugh (1940) showed that spermatozoa are unaffected by hydrostatic pressure.

In view of the successful results obtained with comparatively wide tubing attention was turned to the use of ampoules, and 1 c.cm. of semen was frozen at -196° C. in an ordinary 5-c.cm. pyrex ampoule. On thawing out, numerous active spermatozoa were seen in the sample.

Consideration was then given to more prolonged storage of the vitrified material. Maintenance at -196° C. for any lengthy period was clearly impracticable with the apparatus available. The results given in the Table, however, do not suggest that when 1-mm. tubes are used there is any advantage in vitrifying at -196° C. as compared with -79° C. An experiment extended this conclusion for larger containers, and further showed that transference from -196° C. to -79° C.

had no adverse effect. Ampoules containing 1 c.cm. of semen were therefore vitrified at $-196^{\circ}\text{C}.$ or $-79^{\circ}\text{C}.$ and maintained at the latter temperature for 2 days and 8 days. In each case thawing out showed that an abundance of spermatozoa had survived. A long-term experiment has now been set up, but in view of the work of Jahnel and of Shettles it is fairly certain that under optimum conditions large numbers of human spermatozoa will survive prolonged vitrification.

A much more important and doubtful point is whether vitrification affects the power of the spermatozoon to fertilize and activate the egg and induce normal embryonic development. In the experiments on fowl spermatozoa, quoted above, normal development was not induced after freezing for 1 hour at $-79^{\circ}\text{C}.$, but the partial dehydration before vitrification may have affected their genetic properties. The question is of great interest. Artificial insemination of humans will no doubt become increasingly common, and the elaboration of a method permitting of prolonged storage and transport of the semen without affecting the genetic properties of the spermatozoa would open up remarkable possibilities. It is not obvious how the problem of genetic modification is to be investigated with human material, and it is unfortunate that the spermatozoa of laboratory mammals, including those of the Rhesus monkey, are killed by vitrification under any conditions so far investigated.

Summary

A large proportion of human spermatozoa survive for long periods in semen frozen in bulk at $-196^{\circ}\text{C}.$ or $-79^{\circ}\text{C}.$

Spermatozoa do not survive when minute amounts of semen are frozen as films or in fine capillary tubes.

The rate of freezing and thawing is not the primary factor in the survival of human spermatozoa exposed to low temperatures.

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REFERENCES

- Davenport, C. B. (1897). *Experimental Morphology: Part I, Effect of Chemical and Physical Agents upon Protoplasm*, Macmillan Company, New York.
Hoagland, H., and Pincus, G. (1942). *J. gen. Physiol.*, **25**, 337.
Jahnel, F. (1938). *Klin. Wschr.*, **17**, 1273.
Luyet, B. J., and Hodapp, E. L. (1938). *Proc. Soc. exp. Biol.*, N.Y., **39**, 433.
Marshall, D. A., and Rugh, R. (1940). *Ibid.*, **43**, 141.
Shaffner, C. S. (1942). *Science*, **95**, 337.
Henderson, E. W., and Card, C. G. (1941). *Poultry Science*, **20**, 259.
Shettles, L. B. (1940). *Amer. J. Physiol.*, **128**, 408.

HAEMOLYTIC STREPTOCOCCAL THROAT INFECTIONS: THEIR COMPLICATIONS AND SEQUELS

WITH SPECIAL REFERENCE TO PENICILLIN TREATMENT

BY

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The British garrison in a small Italian harbour town has recently had a very high incidence of carriers of haemolytic streptococci. No systematic survey had been attempted, but a throat swab from any unselected person not showing in the culture at least a few colonies of haemolytic streptococci was an unusual occurrence. Accordingly the number of admissions for haemolytic streptococcal tonsillitis was considerable—79 cases, including 11 peritonsillar abscesses, in 6 months. A few complications such as pneumonia and scarlet fever also occurred. Along with the streptococcal infection ran a high incidence of erythema nodosum and acute rheumatism—some of the cases developing under our very eyes.

The material may be divided in two groups: the first covers the period Nov., 1944, to Jan., 1945, and comprises 34 cases of tonsillitis, including two peritonsillar abscesses, 3 cases of pneumonia, 1 of scarlet fever, 4 of erythema nodosum, and 6 of acute rheumatism; the second group of patients, treated in Feb.–April, 1945, consists of 43 cases of tonsillitis—including 9 peritonsillar abscesses, 7 cases of erythema nodosum, and 11 cases of acute rheumatism.

Acute Tonsillitis

No special attention at first had been paid to the mild cases. If the temperature did not rise above 102° for more than two days no treatment was given. The throat swabs remained positive in almost every case. An attempt to sterilize the throats

in a number of cases through frequent spraying with sulphathiazole powder or calcium penicillin powder failed completely. Cases with pyrexia in the first group were given 20 to 30 g. of sulphathiazole, but the results were no better. Six of the 32 cases returned with a recurrence of their symptoms.

Encouraged by the favourable results achieved by MacGregor and Long (1944) with penicillin pastilles, we introduced them as a routine treatment at the end of January. In preparing the pastilles we followed the technique described by these authors, and gave 10 pastilles daily, 1½-hourly, until the throat became clear. A throat swab was taken on admission, and treatment started at once. Anything between 18 and 46 pastilles was given. On completion of treatment a throat swab was taken again, and the patient was discharged from hospital if it was sterile. No recurrence of symptoms and no complications occurred in this group. The clinical and bacteriological results are summarized in Table I.

TABLE I.—*Haemolytic Streptococcal Tonsillitis*

	Mild Cases (Temperature not above 102° for more than Two Days)				Moderate and Severe Cases (Temperature above 102° for Two or More Days)			
	No Treatment	Sulphathiazole Spray 4-hourly	Calcium Penicillin Spray 4-hourly	Penicillin Pastilles	Sulphathiazole 20–30 g.	Sulphathiazole 20–30 g. and Calc. Penicillin Spray	Sulphathiazole 20–30 g. and Penicillin Pastilles	Penicillin Pastilles only
No. of cases	6	6	6	18	7	7	8	8
Clinical cure in days	4–11	5–10	6–11	3–5	5–21	6–22	3–7	2–9
Average	(7)	(6)	(8)	(3½)	(14)	(13)	(5)	(6)
Days in hospital	7–14	7–16	8–14	5–10	8–33	8–30	5–10	4–14
Average	(10)	(11)	(11)	(8)	(17)	(16)	(8)	(8)
No. of patients with positive cultures before discharge	5	6	5	0	5	7	0	1

No claim of a permanent sterilization can be made. In two cases of chronic tonsillitis throat swabs remained positive after 88 and 126 pastilles.

Peritonsillar Abscesses

The two patients with peritonsillar abscesses in the first group were given 30 g. of sulphathiazole and inhalations. One recovered in 12 days, but his general condition did not allow discharge from hospital until the 28th day. Repeated throat swabs showed pure growths of haemolytic streptococci. The second was very seriously ill for 3 days and a tracheotomy was considered; he recovered slowly, and left hospital after 26 days. The throat swab before discharge still showed a good growth of haemolytic streptococci.

The results in the nine cases of the second group are summarized in Table II.

TABLE II.—*Cases of Haemolytic Streptococcal Peritonsillar Abscesses Treated with Penicillin Pastilles*

Patient No.	No. of Penicillin Pastilles Given	Subjective Relief (Days)	Clinical Cure (Days)	Discharge from Hospital After (Days)	Throat Swab on Discharge
1	22	1	3	8	Sterile
2	18	1	2	8	"
3	40	2	4	9	"
4	28	2	3	10	A few colonies of haemolytic streptococci
5	32	1	3	8	Sterile
6	36	1	4	7	"
7	20	1	2	6	"
8	38	2	4	11	"
9	42	1	4	9	"

Erythema Nodosum

The four cases of erythema nodosum in the first group occurred in Jan., 1945, all in patients with mild haemolytic streptococcal tonsillitis—two untreated and two treated with sulphathiazole spray. In one of each group acute rheumatism followed. The rash faded after 8 to 11 days, during which period throat swabs constantly showed a growth of haemolytic streptococci. The erythrocyte sedimentation rate