two and four months later while still using the ointment, although the organisms remained sensitive to the antibiotic. Two cases were much improved and two showed no response.

Ecthyma.—Of the 10 cases of pyoderma and ecthyma treated, 3 healed in less than one week; these were all cases in which *Staph. pyogenes* alone was cultured. The remaining seven cases had a mixed infection of staphylococci and streptococci, two healing in less than a fortnight and the other five showing no response.

Miscellaneous.—Eight cases of infected skin conditions, including seborrhoeic dermatitis, contact dermatitis, and eczema, were all treated without any sensitization reactions. Three cases healed in less than one week, four in two to three weeks, and one case showed no response. One case of chronic blepharitis healed in three days. Three cases of retro-auricular infective eczematoid dermatitis healed in less than two weeks. The five cases of furunculosis had resisted a variety of treatments for this condition, and only one case ceased to have boils on treatment with neomycin ointment.

Comment

In the treatment of impetigo contagiosa, neomycin ointment is effective and rapid in action. In view of the advantages of using neomycin as a solely local antibiotic it might with advantage be adopted as a routine treatment for this condition.

In this series 60% of cases of impetigo cleared in less than a week, and many of these took only three to four days. Forbes (1952) treated 67 cases of impetigo with neomycin, 62 clearing in less than a week; he remarks that the type of patient was of high standard, most of them being university students and able to follow instructions. It might be assumed that there is some difference in this capacity between American college students and the industrial population of Sheffield. In the treatment of impetigo, chloramphenicol cream (Newman and Feldman, 1951), aureomycin ointment (Solomons, 1951), and bacitracin (Miller *et al.*, 1949) all give comparable results, most cases healing in less than a week, though Solomons found two cases aggravated by aureomycin cream.

In the treatment of sycosis barbae, aureomycin cream seems to have given the best results. Solomons treated 22 cases, all of which responded, only three being left with a few scattered pustules. Chloramphenicol cream (Newman and Feldman, 1951) cured only one case in five, improved two cases, and gave no result in two. Bacitracin (Miller *et al.*, 1949) cured 5 of 16 cases, the other 11 showing no response. Neomycin ointment in this series cured eight cases, and improved five; there was no response in five. Neomycin therefore seems to be superior to all antibiotics except aureomycin in the treatment of this condition.

Prolonged local application of any antibiotic encourages the growth of resistant organisms, and in this series the only aureomycin-resistant staphylococci were found in two cases of sycosis barbae. The routine use of a purely local antibiotic would therefore seem desirable as the first line of attack in the treatment of this condition.

As a local application to infected skin conditions and infective eczematoid dermatitis, neomycin showed no tendency to irritate the skin. Over a large number of cases this harmlessness is unlikely to continue, but Kile *et al.* (1952) applied neomycin to 869 cases and obtained only one sensitivity reaction. In the same paper the authors remark that some patients with haemolytic streptococcal infections did not respond well to neomycin. This was also found in this series in the cases of ecthyma, though impetigo with streptococci responded well. In the cases of sycosis barbae which did not respond or which relapsed the organisms had not developed resistance to the drug. This state is unlikely to last, although it has been the experience of all other investigators of neomycin. Nevertheless, antibiotic therapy is literally becoming a process of hurrying to use the new drug before it ceases to cure.

Summary

Ninety-three cases of pyogenic skin disease were investigated bacteriologically; *Staphylococcus pyogenes* was isolated and found to be resistant to penicillin in 46 cases, to chloramphenicol in 18 cases, and to aureomycin in 2 cases. None were resistant to neomycin.

The cases were treated with neomycin ointment in a strength of 5 mg. per g. In the treatment of impetigo the drug gave results comparable to those of aureomycin, chloramphenicol, and bacitracin. In sycosis barbae the results were superior to those of all other antibiotics except aureomycin. In other skin conditions, with the exception of the deeper infections with haemolytic streptococci, infection was controlled and sensitization reactions were not encountered.

Neomycin ointment is suggested as a routine treatment for impetigo and as the first line of attack in sycosis barbae.

My thanks are due to Dr. J. Colquhoun for the bacteriological investigations. I am indebted to Dr. H. R. Vickers and Dr. I. B. Sneddon for permission to use their cases for this investigation, and to the Upjohn Company for the supply of neomycin and neomycin ointment.

REFERENCES

- Forbes, M. A. (1952). *Sth. med. J.*, **45**, 235.
Kile, R. L., Rockwell, E. M., and Schwarz, J. (1952). *J. Amer. med. Ass.*, **148**, 339.
— Welsh, A. L., and McAfee, G. D. (1950). *Arch. Derm. Syph., Chicago*, **62**, 911.
Kimberly, L. W. (1952). *J. Iowa St. med. Soc.*, **42**, 221.
Livingood, C. S., Nilasena, S., King, W. C., Stevenson, R. A., and Mullins, J. F. (1952). *J. Amer. med. Ass.*, **148**, 334.
Miller, J. L., Slatkin, M. H., and Johnson, B. A. (1949). *Arch. Derm. Syph., Chicago*, **60**, 106.
Newman, B. A., and Feldman, F. F. (1951). *Ibid.*, **64**, 212.
Solomons, B. (1951). *British Medical Journal*, **2**, 525.
Waksman, S. A., and Lechevalier, H. A. (1949). *Science*, **109**, 305.

STORAGE OF TESTICULAR TISSUE AT VERY LOW TEMPERATURES

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The discovery that glycerol has the property of protecting the spermatozoa of certain species (Polge, Smith, and Parkes, 1949; Smith and Polge, 1950) and also red blood cells (Smith, 1950; Sloviter, 1951a, 1951b) against the otherwise fatal effects of freezing and thawing was extended by Smith and Parkes (1951) to ovarian tissue. The latter work was recently recorded in more detail (Parkes and Smith, 1953), and it is clear that rat ovarian tissue, soaked in glycerol-containing media and subsequently frozen slowly and stored in liquid air, suffers only slight loss of its capacity to form an endocrinologically active graft. Moreover, the loss is not increased by prolonging very greatly the period of storage.

Method

Analogous observations have now been made on the preservation and grafting of rat testis tissue. The testes were taken from male rats 7 to 9 days old, at which age the organ is firm and easy to handle. The tunica was slit and the contents were slightly teased out. The capacity of such preparations to form functional grafts, after treat-

ment of various kinds, was tested by homoplastic grafting into the subcutaneous space of the flank in newly castrated adult rats. The tissue was grafted (a) without further treatment, (b) after soaking in 15% glycerol-saline, and (c) after soaking and cooling slowly in saline or 15% glycerol-saline to -79°C . (the temperature of dry ice) or -190°C . (the temperature of liquid air) for various periods, and thawing quickly at $+40^{\circ}\text{C}$., according to the technique described by Parkes and Smith (1953) for ovarian tissue. Groups of 10 animals were used for each experiment. One or more animals in each group were killed at early stages, but the majority were allowed to live for three to four months after grafting, during which time a number of mating experiments were carried out. At necropsy the graft was removed for sectioning and the seminal vesicles were fixed and weighed. The gametogenic activity of the graft was assessed by its histological appearance, and its androgenic activity by the previous mating behaviour of the animal and the size of the seminal vesicles.

The histological development of these and other grafts of frozen testis tissue, especially in relation to spermatogenesis, will be dealt with in a paper by Dr. Deanesly. All that need be said here is that the endocrinologically active grafts recovered during the present series of experiments, whether derived from frozen or unfrozen tissue, resembled those usually obtained when testis tissue is grafted subcutaneously. The tubules were small and the interstitial and other intertubular tissue was prominent. The later stages of spermatogenesis were absent, but some tubules contained spermatocytes in active division. No regular difference was detected between established grafts derived from unfrozen tissue and those from tissue frozen in glycerol-saline.

Results

The experiments recorded here are summarized in the Table, in which the number of animals showing evidence of substantial androgenic activity on the part of the graft

Results of Grafting Rat Testicular Tissue Soaked and Frozen in Saline or 15% Glycerol-saline

Treatment	Time Tissue Frozen	No. of Animals Receiving Grafts	No. Developing Androgenic Grafts
Untreated	—	8	5
Soaked in glycerol-saline	Not frozen	8	5
Soaked in saline and frozen to -79°C .	One hour	6	0
	One hour	8	7
	One week	8	6
Frozen to and stored at -79°C .	8 weeks	5	2
	22 "	9	4
	One hour	16	14
Frozen to and stored at -190°C .	One week	10	8
	7 weeks	6	3
	22 "	9	7

Animals killed 12 to 16 weeks after receiving grafts.

is given in relation to the conditions and duration of freezing. It will be seen that tissue which had been frozen in 15% glycerol-saline and thawed formed an androgenic graft as readily as did unfrozen tissue, and that its viability did not obviously decrease on prolonged storage at low temperature. It appears that the difference between preservation in liquid air and in dry ice is not so marked as with rat ovarian tissue (Parkes and Smith, 1953). In considering the percentage of takes, it must be remembered that grafts showing only slight activity were not included in the right-hand column of the table, and that in all cases the grafts were homoplastic ones of some months' standing. In these circumstances the results with the tissue frozen in glycerol-saline must be regarded as promising.

The entry in the table for the grafting of tissue frozen in saline only should be amplified by recording that of the six rats kept for more than 12 weeks after implantation four developed small grafts, histologically recognizable as rudimentary testis tissue. The seminal vesicles, however, were completely atrophic macroscopically, and histological examination of the vesicular epithelium failed to provide

evidence that the grafts had been even slightly active androgenically. The results were thus in strong contrast to those obtained with testes frozen in 15% glycerol-saline.

It seems, therefore, that the endocrine cells of the testis can be added to the list of tissues the prolonged storage of which at low temperatures is made possible, or greatly facilitated, by the use of media containing glycerol. It remains to be seen to what extent this technique may promote the use of viable transplants of endocrine and other tissue in man. Discussion of this problem will be found in the forthcoming volume of the Proceedings of the recent Ciba Symposium on the Preservation of Normal Tissues for Transplantation.

The operations and day-to-day observations were carried out by Miss J. Masson and Miss M. Mansi.

Summary

The androgenic tissue of the testis of the young rat can be preserved in viable condition for long periods by freezing in media containing glycerol, as previously described for the ovary of the rat (Smith and Parkes, 1951).

REFERENCES

- Parkes, A. S., and Smith, A. U. (1953). *Proc. roy. Soc. B*, **140**, 455.
 Polge, C., Smith, A. U., and Parkes, A. S. (1949). *Nature, Lond.*, **164**, 666.
 Sloviter, H. A. (1951a). *Lancet*, **1**, 823.
 — (1951b). *Ibid.*, **1**, 1350.
 Smith, A. U. (1950). *Ibid.*, **2**, 910.
 — and Parkes, A. S. (1951). *Ibid.*, **2**, 570.
 — and Polge, C. (1950). *Vet. Rec.*, **62**, 115.

Medical Memoranda

Intolerance to "Veriloid"

"Veriloid," a preparation of the alkaloids of *Veratrum viride* or green hellebore, is a potent hypotensive agent. Alarming symptoms from its use in moderate dosage occurred in the following case.

CASE REPORT

A married woman aged 59, living alone, was admitted to Whittington Hospital at 9 p.m. on August 14, 1952, complaining of severe dull epigastric pain radiating upwards into the neck and down both arms; it had come on at 5 p.m. the same day. At the onset she had felt choked, vomited, and had some blurring of vision. She had been unable to get to her doctor for some time.

On examination she looked ill and her extremities were cold. The pulse rate was 40, regular, and the blood pressure was 80/60. The apex beat was displaced a little to the left; the heart sounds were normal. The fundi showed only slight arterial changes. There was no excessive salivation, and the pupils were of normal size. No abnormality was found in the lungs, abdomen, or central nervous system.

A provisional diagnosis of myocardial infarction was made, and she was given morphine $\frac{1}{2}$ gr. (11 mg.). The pulse rate did not rise above 60, and the blood pressure remained at 100/60 or less. The symptomatology was then reconsidered, and veriloid overdosage was thought more likely to have been the cause. She was therefore given atropine, 1/150 gr. (0.43 mg.). Her pulse increased slightly to 64, her blood pressure rose only to 115/80, but her pain ceased in the next hour and a half. At this point ephedrine, $\frac{1}{2}$ gr. (32 mg.) was given by intramuscular injection. One hour later her pulse rate was 74 and her blood pressure 165/100. Electrocardiograms on August 15, 19, and 26 showed no abnormality. The white-cell count was 7,700 with a normal differential count, and the sedimentation rate was 8 mm. in one hour.

She remained well, up and about in the ward, with no return of pain or distress, from August 16 to 30, when she was discharged. Her resting blood pressure was then 200/110.