and with several turns of the trephine the corneal epithelium is cut. The trephine and marking-ring are now removed and the trephine cut is stained with fluorescein.

The acrylic splint is now placed on the cornea so that it overlaps the trephine incision, which is seen through the transparent acrylic as a green ring. The flanges are placed in the 12 to 6 o'clock meridian and the 9 to 3 o'clock meridian. The cornea is dried with the tip of a sterile filter paper at the sites of the notches in the flanges, and these sites are marked on the cornea with a dot of gentian violet.

The acrylic splint is now removed. No. 000 black silk sutures threaded at each end with Grieshaber's needles are passed through half the thickness of the cornea at the site of the dots; in the case of the 12 and 6 o'clock flanges the sutures pass transversely and in the 9 and 3 o'clock positions they pass vertically (Fig. 3). The arms of these

FIG. 3.—Suture anchorage of acrylic splint to the cornea and the conjunctiva at the limbus.

sutures are then secured to the head towel with bulldog clips in their proper order.

The disk of opaque cornea is now cut with a 5.1-mm. trephine and removed. Any tags of Descemet's membrane are trimmed. The corneal graft is set into its bed and the acrylic splint is placed over this. The edges of the graft are clearly seen through the transparent acrylic, and any necessary adjustment may be made with the tip of an iris repositor. The notches of the flanges are placed in line with the sutures, which are now brought forward in the notches and tied with a surgical knot, the needles being retained on the ends of the sutures.

To make the anchorage of the acrylic splint even more secure each arm of the suture is brought out through the conjunctiva at the limbus at a site about equidistant from each flange (Fig. 3). For instance, in the right eye the temporal arm of the 12 o'clock flange suture is carried through the conjunctiva at 10.30 o'clock and so is the upper arm of the 9 o'clock flange suture. These sutures are then tied and their ends left long. A like procedure is done with the nasal arm of the 12 o'clock flange suture and the upper arm of the 3 o'clock flange suture, which traverse the limbal conjunctiva at 1.30 o'clock, and so with the other sutures to effect additional anchorage at 7.30 and 4.30 o'clock in the limbal conjunctiva. The ends of these sutures are left long and the pairs of these are then brought obliquely across the acrylic splint from 7.30 to 1.30 and

4.30 to 10.30 o'clock. This cross of double sutures is the third means of anchoring the acrylic splint.

Post-operative Technique.—Between the eighth and tenth days after operation one or two of the limbal sutures cut out, but the corneal sutures hold firm. The acrylic splint is removed on the tenth day. In seven cases thus treated to date, the graft has been clear, its edge showed perfect coaptation to the bed in the recipient's cornea, and its surface was evenly moulded by the splint to conform with the curvature of the cornea. There has been some conjunctival irritation, particularly at the limbus.

Summary

A method of fixing a corneal graft by means of an acrylic disk splint with four flanges anchored to the cornea with sutures is described.

Acknowledgment for the use of Fig. 1 is made to Messrs. John Wright, of Bristol, who published it in the second edition of *Eye Surgery*, and for Fig. 2 to the Editor of the *British Journal of Ophthalmology*.

EFFECTS OF PENICILLIN AND STREPTOMYCIN ON VACCINE LYMPH

BY

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With the best of care, heavy bacterial contamination of vaccine lymph (calf lymph) is inevitable during its preparation, and as many as 500 million organisms per ml. may be present, particularly in the Tropics. They belong mostly to the cocci group, but may include also *Bacillus subtilis*, *Bact. coli*, *Pseudomonas pyocyanea*, yeasts, and fungi; anaerobic organisms may also be occasional contaminants. None of the methods so far advocated to reduce the number of bacteria can be considered satisfactory. The use of eggs and tissue-culture to prepare the sterile vaccine aseptically has not met with universal approval, because of the uncertain immunizing power of the product: calf lymph still holds the field. Many institutes in India employ what is known as the "chloroform process," in which chloroform vapour is bubbled through the vaccine lymph for a known time to sterilize it. But, since chloroform has no action on spores, the spore-bearers present in the lymph persist. Other countries use different methods. But so far no method has been found consistently to give absolute bacterial sterility.

The antibiotics, by virtue of their action on bacteria, should be of great value in the purification of vaccine lymph; and I have made a detailed study of their use under different conditions.

Effect of Penicillin on Glycerinated Vaccine Lymph

This has already been investigated by various workers (Diaz Romero, 1945; Morin and Turcotte, 1946; Patel, 1948) with very encouraging results, but in my experience it is disappointing (Krishnamurthy, 1949). This is probably because previous work seems to have ignored the possibility of interference with the sterility tests by the residual penicillin in the stored lymphs. In the present experiments residual penicillin from the treated lymph was removed by alternate washing with distilled water and centrifuging four times, and the sediment was tested for sterility. The plate showed numerous colonies of staphylococci and a few

B. subtilis groups of organisms, while the unwashed lymph (still containing penicillin) naturally gave no growth whatever (Table I).

TABLE I.—Effect of Penicillin

No.	Penicillin (Units per ml.)	Duration of Contact with Penicillin	Colony Count on Lymph Samples	
			Before Removal of Penicillin	After Removal of Penicillin
1	500	1 week	No growth	230 millions per ml.
2	5,000	1 "	"	238 "
Control	Nil (untreated lymph)		240 millions per ml.	" "

0.1 ml. of undiluted lymph was used for testing unwashed penicillin-treated lymphs and 1 ml. of one in a million dilution was used for penicillin-removed and untreated control lymphs.

It is clear that the organisms are not killed but merely inhibited by penicillin, and once the penicillin is removed or inactivated the inhibited organisms show up.

Effect of Streptomycin on Glycerinated Vaccine Lymph

In one of the experiments streptomycin was added in concentrations varying from 500 μ g. to 10,000 μ g. per ml. of glycerin vaccine lymph and preserved in cold storage. Sterility tests after varying periods gave encouraging results. It was observed that there was a fall in the bacterial population proportional to the concentration of streptomycin and the duration of its contact (Table II).

TABLE II.—Effect of Streptomycin

Sl. No. of Lymph	Conc. of Streptomycin (μ g. per ml.)	Staphylococcal Colony Count per ml. After	
		24 Hours	1 Week
1	500	10,200	7,900
2	1,000	1,400	1,100
3	2,000	300	100
4	5,000	240	40
5	Nil	252 millions	240 millions

No appreciable difference was noted between tests done before and after removal of residual streptomycin by alternate washing with distilled water and centrifuging six times, except that there was some growth of *B. subtilis* afterwards, showing that the spores were only inhibited and not killed by the antibiotic.

In combination with 500 units of penicillin per ml. as little as 500 μ g. of streptomycin per ml. reduced the bacterial contamination to a greater extent (Table III).

TABLE III.—Effect of Combined Action of Streptomycin and Penicillin

Sl. No.	Conc. of Penicillin (Units per ml.)	Conc. of Streptomycin (μ g. per ml.)	Colony Count per ml. After	
			24 Hours	1 Week
1	500	500	150	60
2	1,000	1,000	130	60
3	2,000	2,000	140	30
4	Nil	Nil	252 millions	240 millions

Tests were done after removing the residual antibiotics to eliminate penicillin interference. *B. subtilis* spores were found to be unaffected by this method also.

A study of the effects of these antibiotics under conditions optimal for their action, permitting a growth phase of the bacterial flora of the vaccine, was next taken up.

Effects of Antibiotics on Vaccine Pulp at 37° C.

Vaccine material (vaccine pulp) immediately after collection from the calf was diluted with two volumes of distilled water (no glycerin was added) and well emulsified. The emulsion was then treated with streptomycin

in concentrations varying from 500 μ g. to 10,000 μ g. per ml., both singly and in combination with penicillin, and incubated at 37° C. for 24 hours. Bacterial reduction was complete after 24 hours' incubation in 5,000 μ g. of streptomycin per ml. singly or in combination with 1,000 units of penicillin. *B. subtilis* spores were also affected. It was found that moulds which persisted in lower concentrations were suppressed in 10,000 μ g. of streptomycin (Table IV).

TABLE IV.—Effect of Streptomycin and Penicillin on Vaccine Emulsion (Without Addition of Glycerin) at 37° C. for 24 Hours

Sl. No. of Samples	Conc. of Streptomycin (μ g. per ml.)	Conc. of Penicillin (Units per ml.)	Bacterial Count per ml.
1	500	—	1,800 staph.
2	1,000	—	1,500 staph.
3	5,000	—	Moulds only
4	10,000	—	Nil
5	—	500	Numerous <i>Bact. coli</i> and 250 staph.
6	—	1,000	Numerous <i>Bact. coli</i> and 200 staph.
7	500	500	150 staph.
8	1,000	1,000	120 staph.
9	1,000	5,000	Moulds only
10	1,000	10,000	Nil
Control	—	—	1,200 millions (staph., <i>B. subtilis</i> , <i>Bact. coli</i> , and moulds)

Tests were done after removal of residual antibiotics.

Penicillin alone in concentrations of 500 and 1,000 units per ml. under the same conditions brought down the staphylococcal contaminants to 250 per ml. and 200 per ml., respectively, and the *B. subtilis* content to nil, but allowed an abundant growth of *Bact. coli* (Table IV).

External Application to the Calf

Application of streptomycin in combination with penicillin in varying concentrations over the vaccinated area of the calf twice a day for five days also yielded good results. Three different concentrations (100 units of penicillin and 1,000 μ g. of streptomycin per ml., 500 units of penicillin and 5,000 μ g. of streptomycin per ml., and 100 units of penicillin and 10,000 μ g. of streptomycin per ml.) were used on different animals. The first application was about one hour after vaccinating the animal, and the last one about 18 hours before the scraping of the vesicles. The scraped material was emulsified in glycerin in the usual manner and stored in the refrigerator. It was interesting to find that external application of streptomycin reduced the bacterial contamination to a very great extent, the count being not more than 200 organisms per ml. of vaccine lymph in all the cases. *B. subtilis* spores were unaffected by this method also.

Effects on Potency of Lymph

Vaccine lymphs treated with different concentrations of the two antibiotics for varying periods were tried periodically for potency on rabbits. The highest concentration of penicillin used was 10,000 units per ml., with a contact period of about one year, and of streptomycin 10,000 μ g. per ml., with a contact period of about six months. None of the samples tested showed any loss in potency.

Clinical Trial on Children.—Penicillin-treated lymphs were not tried on human beings, on account of their bacterial impurity. A preliminary trial, on children, of streptomycin-treated lymphs (both with and without further dilution) satisfying the various standardization tests indicated that the vaccination "takes" were normal and uneventful. The work is being pursued further.

Discussion

Chain and Duthie (1945), Todd (1945), and others have established that penicillin is bactericidal against susceptible

organisms only during their growth phase in a nutrient medium and not while "resting" in a non-nutrient fluid. Glycerinated vaccine lymph is a non-nutrient medium, which explains the failure of penicillin in reducing the bacterial flora.

On the other hand, Strauss (1947) and Garrod (1948) have clearly demonstrated that streptomycin is bactericidal both in nutrient and in non-nutrient fluids, though needing higher concentrations in the latter; and streptomycin has likewise proved effective in lymph. Further, it acts synergistically with penicillin, as little as 500 µg. of streptomycin with 500 units of penicillin per ml. producing an equally effective bacterial reduction of vaccine lymph. Chain and Duthie (1945), Himmelweit (1945), and Pulaski, Kleinman, and Duncan (1946) report that certain antibiotics in combination with sulphonamides, bacteriophage, or other antibiotics have such a synergistic action.

While the Therapeutic Substances Act permits up to 5,000 organisms per ml. of lymph, streptomycin-treated lymphs have barely 200 organisms per ml.—in some cases no bacteria at all. The minimal requirement for a consistently successful vaccination "take" in children in the Tropics is a 10^{-4} or 10^{-5} positive potency (as tested by continuous vaccinal vesiculation on the rabbit); the streptomycin-treated lymphs have usually a 10^{-8} positive potency, or more. This permits the use of highly diluted vaccines and effects a great saving in production cost.

Summary

The effects of penicillin and streptomycin on vaccine lymph (calf lymph) under different conditions are described.

Penicillin is ineffective in reducing the bacterial flora of vaccine lymph under those conditions; this becomes evident only when tests are conducted after removal of residual penicillin from the penicillin-treated lymphs.

Streptomycin successfully brings down the bacterial flora of the vaccine lymph to the required extent.

Neither antibiotic affects the potency of vaccine lymph.

The application of these findings to the preparation of purer calf lymph at a substantially lower cost than at present is discussed.

I am grateful to Dr. T. Chandrasekhariah, Director of Public Health in Mysore, for granting the facilities to undertake this work and for permission to publish this paper. I wish also to thank my staff for their co-operation throughout.

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The British Hospitals Contributory Schemes Association combined its second annual general meeting in London on October 13 with a conference on convalescence and aftercare opened by Sir Allen Daley, chief medical officer of London County Council. Mr. Henry Lesser was reappointed president, Mr. W. G. Nevin honorary treasurer, and Mr. John Dodd honorary secretary. Three million citizens are now raising £2m. a year to obtain benefits supplementary to the National Insurance and Health Service Acts, and the thirty schemes affiliated to the association raised charitable income for amenities for hospital patients, medical research, and medical charities of more than £40,000 in 1949.

Medical Memoranda

Cushing's Syndrome Presenting with Features of a "Type II" Nephritis

The following case is interesting enough to be placed on record.

CASE REPORT

A 3-para aged 41 was admitted on January 3, 1949, complaining of swelling of face, abdomen, and lower limbs of two months' duration. There was some frequency of micturition and thirst.

On admission she was found to be obese, with gross, very soft pitting oedema of lower limbs and abdomen; hair growth was normal; blood pressure 190/90; fundi were normal. The urine contained a "light cloud" (40–80 mg./100 ml.) of albumin; no casts; no sugar; protein, 0.252 g. per 100 ml.; total protein in 24 hours, 3.8 g. The blood urea content was 29 mg. per 100 ml.; blood urea clearance, 68% of average normal; plasma total protein, 4.6 g.% (albumin 3.1 g., globulin 1.3 g., fibrinogen 0.2 g.); A/G ratio, 2.4:1. Serum cholesterol, 408 mg./100 ml.

The presumptive diagnosis was Type II nephritis (Ellis), and she was put on a salt-free high-protein diet. After three weeks the oedema had almost subsided. There was only a trace of albumin in her urine (0–2 mg./100 ml.), and she felt better. Her blood pressure was still 190/90. She was discharged and told to attend the out-patient department for a follow-up.

The patient was readmitted on February 16. She had been getting weaker for three weeks, suffering from intense thirst, anorexia, constipation, and mental depression, and had missed two periods.

On examination she was ill, lethargic, and dehydrated. There was a dusky cyanosis of her rather large face, which was covered with fine downy hair. The skin throughout was dry and scaly, and there was generalized pitting oedema, most noticeable in the legs. The external primary and secondary genitalia were atrophic. Blood pressure was 185/105; fundi were normal. A firm mass could be palpated in her right loin as well as a firm and possibly irregular liver edge. Cutis marmorata was noticed. A diagnosis of Cushing's syndrome was made, with a suprarenal lesion and some renal defect.

Investigations after readmission showed: blood urea, 58 mg./100 ml.; urine, 40–80 mg. of albumin per 100 ml.; very few hyaline and granular casts; haemoglobin, 73%; R.B.C., 4,270,000; C.I., 0.85; serum cholesterol, 200 mg./100 ml.; 17-ketosteroids per 24 hours, 31 mg.; blood sodium, 335 mg./100 ml.; blood potassium, 16.5 mg./100 ml.; plasma phosphorus, 1.2 mg./100 ml.; serum calcium, 8.7 mg./100 ml.; urine loaded with sugar and acetone (to 2%); blood sugar content, 407 mg./100 ml.

After stabilization of her diabetes Mr. Donald Barlow, on April 5, removed a large encapsulated suprarenal tumour. In spite of pre- and post-operative saline plasma, "eucortone," deoxycorticosterone acetate, "methedrine," etc., the patient died 14 hours after the operation.

Post-mortem findings showed a typical adrenal cortical carcinoma with secondary deposits in the liver and lungs. Macroscopically the kidneys, thyroid, and pituitary gland were normal. The left adrenal was smaller than normal. Microscopically the kidneys showed some tubular cloudy swelling, but the glomeruli gave no evidence of nephritic change. True basophil adenoma was not found in the pituitary, but there was an excess in the numbers of basophil cells present.

COMMENT

The interesting feature of this case is that the oedema and albuminuria were so pronounced that it was at first assumed to be an example of "Type II" nephritis.