lesser omentum was exactly similar to that in our previous case, we feel that an extrahepatic duct must have been torn.

A partial or complete tear may occur in the extrahepatic ducts which may or may not be obvious at the time of operation. Many forms of surgical treatment have been advocated. Waugh (1916) had a case, very similar to our first, in which two laparotomies were carried out for upper abdominal distension and signs of obstructive jaundice and in which the condition suddenly cleared up spontaneously. Benson and Prust (1953) treated a case successfully by marsupialization and drainage of a false cyst of biliary ascitic fluid which had formed. Gross (1953) had two cases; one was treated successfully by simple drainage from the site of the injury, while in the other an attempt was made at primary repair. Stenosis of the common bile duct, however, developed, necessitating cholecysto-duodenostomy, which led to cholangitis, cirrhosis, bleeding oesophageal varices, and death. Mason *et al.* (1954) had a case which developed stenosis requiring cholecysto-duodenostomy even though no attempt at repair had been made at the primary operation. Hicken and Stevenson (1948) advocated a cholecystostomy to decompress the biliary system, and suggested that cholangiograms could be taken later to demonstrate healing of the tear and also to make sure that any associated traumatic pancreatitis which might obstruct the lower end of the common bile duct had settled before removing the tube.

It is quite clear that injuries to the extrahepatic ducts often heal spontaneously without the development of stenosis, and it is possible that an attempt to repair a recently traumatized duct may increase the likelihood of stenosis. Simple abdominal drainage, and possibly cholecystostomy, should be performed initially; if stenosis develops it can be treated later.

Summary

Two cases of rupture of the extrahepatic bile ducts are described in which the lesion was not recognized at operation. Biliary ascites with minimal systemic upset developed in one, necessitating frequent aspiration. In the other a drainage tube allowed the bile to escape externally. Spontaneous healing of the fistula occurred in both.

REFERENCES

- Benson, C. D., and Prust, F. W. (1953). *Surg. Clin. N. Amer.*, 33, 1187. Saunders, Philadelphia and London.
Gross, R. E. (1953). *The Surgery of Infancy and Childhood*, p. 538. Philadelphia.
Harkins, H. N., Harmon, P. H., and Hudson, J. (1936). *Arch. Surg. (Chicago)*, 33, 576.
Hicken, N. F., and Stevenson, V. L. (1948). *Ann. Surg.*, 128, 1178.
Mason, L. B., Sidbury, J. B., and Guiang, S. (1954). *Ibid.*, 140, 234.
Waugh, G. E. (1916). *Brit. J. Surg.*, 3, 685.

The General Register Office's analysis of the occupations of persons in England and Wales included in the 1951 Census has been published as *Census 1951, England and Wales, Occupational Tables* (H.M.S.O., price seven guineas). It is based on the full figures, thus replacing the results of the 1% sample published in 1952. The findings are compared with those of the 1931 Census. Owing to the difference in school-leaving age at the times of the Censuses, the 1931 occupational figures include some 285,000 children under 15, while the 1951 figures relate to those over that age. In 1951 14 million of the 16 million males were gainfully occupied, and 6½ million of the 18 million females. Of the occupied men, 29% were under 30 and only 4% over 65; of the occupied women, 44% were under 30 and 5% over 60. In 1951 there were 11 million married women, of whom 22% were gainfully occupied compared with 10% in 1931. The two commonest occupations for the married women were as charwomen and clerks—149,000 of each.

THE ANTIBACTERIAL ACTIVITY OF CHLOROPHYLL

BY

SHEILA MOWBRAY, B.A.

*From the Wright-Fleming Institute of Microbiology,
St. Mary's Hospital Medical School, London, W.2*

Numerous reports have been published describing the successful treatment of chronic infected wounds with water-soluble chlorophyll preparations. In many cases healing has been induced where there had been no response to conventional treatment. Bowers (1947) treated 400 various surgical cases with water-soluble chlorophyll and noticed that infected wounds became free from suppuration and odour within two or three days. Combes, Zuckerman, and Kern (1952) produced a similar reduction in suppuration in secondarily infected ulcers, contact dermatitis, and pemphigus. Carpenter (1949) observed an early eradication of infection and early healing in 127 cases of suppurative osteomyelitis and chronic ulcers: the healthy appearance of granulation tissue under chlorophyll therapy was stressed.

Whereas chlorophyll derivatives in wound healing are generally regarded as promoters of healthy granulation, some clinical reports suggested that they have antibacterial properties. Smith (1944) observed *in vitro* bacteriostasis of staphylococci, streptococci, and the anaerobic spore-bearers, while Nevin and Bibby (1954) showed that chlorophyll inhibited the growth of staphylococci, streptococci, and lactobacilli.

The present experiments were designed to investigate further, by *in vitro* methods, the antibacterial activity of a chlorophyll preparation.

Material and Methods

The water-soluble chlorophyll preparation used throughout these experiments was sodium potassium copper chlorophyllin (which is the base of "chloresium" preparations) kindly supplied by Rystan Company, New York. A fresh 10% solution was prepared daily in sterile distilled water, and dilutions of this used in all experiments.

The sensitivity of various organisms to chlorophyll was determined, using a plate method modified from that described by May and Morley (1952). Levinthal agar was used throughout. All organisms were grown in nutrient broth for five hours and the standard inoculum was one loopful, approximately 10^8 organisms. Plates were incubated aerobically for 16 hours at 37° C. The highest concentration of chlorophyll incorporated in solid media was 1/100. This was governed by the solubility of the preparation; a 10% solution was the maximum concentration possible.

Chlorophyll sensitivity was also determined by making bacterial counts of organisms growing in fluid media. Growth curves were plotted by the method of Miles, Misra, and Irwin (1938), with organisms grown in nutrient broth containing dilutions of chlorophyll.

Results

Sensitivity of Various Organisms to Sodium Potassium Copper Chlorophyllin.—The results of the plate method are shown in Table I. Chlorophyll was found to inhibit the growth of some Gram-positive bacteria, but had no effect on the Gram-negative organisms tested. No change in antibacterial properties was produced by heating the chlorophyll at 100° C. for one hour. The presence of 10% horse serum in the medium did not affect the inhibitory level. Since *Staph. aureus* is a common wound contaminant it was chosen as the test organism for all further experiments.

TABLE I.—Sensitivity of Various Bacteria to Chlorophyll

Organism	Inhibitory Level of Chlorophyll	Organism	Inhibitory Level of Chlorophyll
<i>Staph. aureus</i> ..	1/800	<i>Cl. welchii</i> ..	1/200
<i>Staph. albus</i> ..	1/800	<i>Cl. oedematiens</i> ..	1/100
<i>Str. pyogenes</i> ..	1/1,600	<i>Cl. sporogenes</i> ..	1/100
<i>Str. viridans</i> ..	not at 1/100	<i>Cl. tetani</i> ..	1/100
<i>Str. faecalis</i> ..	not at 1/100	<i>Ps. pyocyanea</i> ..	not at 1/100
<i>Pneumococcus</i> ..	1/1,600	<i>Pr. vulgaris</i> ..	not at 1/100
<i>B. pumilis</i> ..	1/800	<i>E. coli</i> ..	not at 1/100

Sensitivity of Different Strains of *Staph. aureus*.—Thirteen strains of *Staph. aureus* were streaked over chlorophyll-Levinthal agar: five were inhibited at a chlorophyll concentration of 1/800, seven at 1/400, and one at 1/100.

Effects of Age of Culture, Time of Inoculation, and Size of Inoculum.—Cultures of one strain of *Staph. aureus* incubated for 2, 4, 6, 8, and 24 hours were plated out on chlorophyll-Levinthal agar. A dilution of 1/800 chlorophyll inhibited growth after 16 hours' incubation in every case. After 24 hours' further incubation *Staph. aureus* grew in the presence of all concentrations of chlorophyll. It appears, therefore, that either chlorophyll is inactivated at 37° C. or it does not completely inhibit growth. Chlorophyll-Levinthal agar maintained at 37° C. for 24 hours before use still inhibited growth for 16 hours at a concentration of 1/800. Hence growth of the test organism after long incubation periods is not due to inactivation of the chlorophyll. The organisms are temporarily inhibited, but after 24 hours bacterial multiplication proceeds. The size of inoculum, ranging from 10⁶ to 10⁸ organisms, did not affect the inhibitory level.

Growth of *Staph. aureus* in the Presence of Chlorophyll.—The growth curves shown in Fig. 1 confirmed the conclusion drawn from the results of the plate method that chlorophyll

FIG. 1.—Growth curves of *Staph. aureus* in nutrient broth containing various concentrations of chlorophyll. n_t = Number of organisms per ml. at time t . n_0 = Number of organisms per ml. at start of experiment.

temporarily inhibits the growth of *Staph. aureus*. Some organisms were killed, the number increasing with chlorophyll concentration, but even when this was 1/20 bacterial multiplication began after 24 hours. Thus it seems that *Staph. aureus* became resistant to chlorophyll. The emergence of chlorophyll resistance could not be demonstrated by the usual methods because the solubility of the preparation is such that a concentration completely inhibiting the organism for indefinite periods could not be used. However, *Staph. aureus* was grown on Levinthal agar containing 1/100 chlorophyll by incubating at 37° C. for 48 hours. After eight subcultures of successively reduced incubation periods the organism grew well in the presence of 1/100 chlorophyll after 16 hours at 37° C. It therefore seems that some form of resistance to chlorophyll is acquired.

Copper Content of Sodium Potassium Copper Chlorophyllin.—The copper content of the chlorophyll preparation

used was 4.16%, and it was thought that its bacteriostatic properties might be due to the copper present. Three different organisms were cultured on two batches of Levinthal agar, one containing dilutions of chlorophyll and one containing copper sulphate in concentrations such that the two batches of medium contained identical amounts of copper. The results are shown in Table II. It may be concluded that the antibacterial properties of chlorophyll are not due to its copper content.

TABLE II.—Comparison of Sensitivity of Three Organisms to Chlorophyll and Copper Sulphate

Organism	Inhibitory Level of Chlorophyll	Inhibitory Level of CuSO ₄ *
<i>Staph. aureus</i> ..	1/800	> 1/100
<i>Staph. albus</i> ..	1/800	> 1/100
<i>Str. pyogenes</i> ..	1/1,600	1/200

* Expressed as concentration of chlorophyll containing same amount of copper.

Effect of Chlorophyll on Bacteriostasis Produced by Antibiotics

The bacteriostatic effect of chlorophyll is slight, and it was thought that it might be better demonstrated by the use of synergism, as in the method described by Dye, Lynch, Cullen, and Sprague (1952). They increased the sensitivity of the agar diffusion assay of viomycin by incorporating sulphadiazine in the medium. The possible potentiating effect of antibiotics on the antibacterial activity of chlorophyll was investigated, using a routine plate assay method. Two-layer plates were poured. Both layers contained a subinhibitory level of penicillin, and the surface layer was seeded with an inoculum of an overnight *Staph. aureus* culture sufficient to produce confluent growth. Six porcelain cylinders were placed on the surface of each plate and were filled with equal volumes of different dilutions of chlorophyll. The plates were incubated for 16 hours and the diameters of the zones of inhibition around each cylinder were measured. The square of the diameter of the zone of inhibition was plotted against the log. of the concentration of chlorophyll.

Subinhibitory concentrations of penicillin incorporated in the medium were found to increase only slightly the zones of inhibition of growth of *Staph. aureus* produced around porcelain cylinders containing chlorophyll. The small size of zone of inhibition around a chlorophyll solution is probably due in part to the low diffusibility of the preparation; the green colour does not extend far beyond the porcelain cups. Hence the two substances were reversed; a subinhibitory concentration of chlorophyll was incorporated in the medium, and penicillin dilutions were put in the porcelain cylinders. A marked difference was observed in zone diameter between those produced on assay media containing chlorophyll and those on straight assay media. The same effect was demonstrated with oxytetracycline ("terramycin") and streptomycin, using *B. pumilis* as the test organism for oxytetracycline and *Staph. aureus* for streptomycin.

These findings are demonstrated graphically in Figs. 2, 3, and 4. It is obvious that subinhibitory concentrations of chlorophyll in the medium cause a vertical shift in the dose-response curves for penicillin, streptomycin, and oxytetracycline.

Growth curves were plotted of *Staph. aureus* growing in nutrient broth in the presence of penicillin alone, chlorophyll alone, and combinations of the two. No synergistic effect could be demonstrated as in the agar diffusion method. Therefore it appears that the increase in sensitivity of the agar diffusion assay of antibiotics produced by subinhibitory concentrations of chlorophyll is due to some physical property of the preparation and is not true synergism. Chlorophyllin is a surface-active agent, and may, by virtue of this property, increase the sensitivity of organisms to antibiotics under the conditions of an agar diffusion assay. Smith and Livingston (1945) found that experimental wounds

treated with chlorophyll and antibiotics healed more quickly than when treated with either agent alone. This effect may partly be due to an enhancement of the antibiotic's activity by the surface active properties of the chlorophyll.

FIG. 2.—Inhibition of *Staph. aureus* by penicillin in the presence of three concentrations of chlorophyll.

FIG. 3.—Inhibition of *Staph. aureus* by streptomycin in the presence of three concentrations of chlorophyll.

FIG. 4.—Inhibition of *B. pumilis* by oxytetracycline in the presence of three concentrations of chlorophyll.

Summary

Soluble sodium potassium copper chlorophyllin (which is the base of "chloresium" preparations) shows some bacteriostatic action against certain Gram-positive organisms, including *Staph. aureus*, *Str. pyogenes*, the pneumococcus, and the pathogenic clostridia. After a period of bacteriostasis, bacterial multiplication proceeds in the presence of all concentrations of chlorophyll tested.

After repeated culture in the presence of chlorophyll *Staph. aureus* becomes resistant to the temporary bacteriostatic effect of this substance.

The activity of some antibiotics is potentiated by the presence of subinhibitory concentrations of chlorophyll.

This work was carried out during the tenure of a research grant from Rystan Company, New York.

REFERENCES

- Bowers, W. F. (1947). *Amer. J. Surg.*, 73, 37.
 Carpenter, E. B. (1949). *Ibid.*, 77, 167.
 Combes, F. C., Zuckerman, R., and Kern, A. B. (1952). *N.Y. St. J. Med.*, 52, 1025.
 Dye, W. E., Lynch, H. P., Cullen, T. M., and Sprague, E. M. (1952). *Antibiot. and Chemother.*, 2, 610.
 May, J. R., and Morley, C. W. (1952). *Lancet*, 1, 636.
 Miles, A. A., Misra, S. S., and Irwin, J. O. (1938). *J. Hyg. (Camb.)*, 38, 732.
 Nevin, T. A., and Bibby, B. G. (1954). *J. dent. Res.*, 33, 571.
 Smith, L. W. (1944). *Amer. J. med. Sci.*, 207, 647.
 — and Livingston, A. E. (1945). *Amer. J. Surg.*, 67, 30.

Medical Memoranda

Rupture of the Uterus Involving the Bladder

Ruptured uterus involving the bladder is a very rare condition. It is more likely to be met with after a previous caesarean section in which the bladder is firmly adherent to the uterine scar. The following case is of unusual interest in that the patient had had no previous operation and gave no history to indicate that the bladder might be adherent to the lower uterine segment. Nevertheless she sustained an extensive tear of the uterus and the bladder during labour, which resulted in the baby's shoulder being lodged in the bladder cavity.

CASE REPORT

The patient, a Yoruba of Western Nigeria aged about 36, was admitted into Massey Street Maternity Hospital, Lagos, Nigeria, on November 16, 1954, with a history of nine months' amenorrhoea and labour pains which had started 18 hours previously. She was a gravida 4, para 3, one alive; the last confinement being three years before admission. Previous pregnancies and deliveries had been uneventful. Her general condition on admission was fair, although she appeared dehydrated. Her temperature was 99° F. (37.2° C.), pulse 120, respirations 24, and B.P. 130/70.

The bladder appeared to be full and was palpable up to the level of the umbilicus. The fundus uteri was level with the ensiform cartilage. The abdomen was very tense and the foetal parts were difficult to palpate. The foetal heart was not heard. Vaginal examination revealed slight bleeding, an almost fully dilated os, with a thick rim of cervix felt posteriorly, and the presentation was a face jammed in mid-cavity. The suspicion of ruptured uterus was confirmed when blood escaped during an attempt to pass a catheter by displacing the head upwards.

At operation blood-stained fluid was found in the abdominal cavity. The bladder peritoneal reflection was raised, dark purplish, and oedematous. When this was opened blood clots were exposed and a longitudinal tear in the lower uterine segment was seen. On reflecting the bladder from the uterus the baby's shoulder came into view and the glistening mucous lining of the bladder was observed. It then became apparent that the bladder was involved in the rupture.

Examination of the damage revealed a longitudinal tear in the lower uterine segment and a T-shaped tear in the bladder, the longitudinal arm of which extended downwards, splitting the trigone of the bladder symmetrically in two and involving the upper third of the urethra.

A subtotal hysterectomy was performed and the bladder repaired with a No. 0 chromic catgut suture. The patient's condition was satisfactory throughout and she appeared very little shocked in spite of the extensive damage. An indwelling catheter was inserted and the abdomen drained. The patient made an uninterrupted recovery but developed