on bacteria, with special reference to the optimal dilution for use on the skin. Observations were mainly made on *Staph. pyogenes* (usually the Oxford strain) and *Str. pyogenes* (Milne strain and local strains), but two tests were performed using a strain of *Ps. pyocyanea*. Six experiments, employing three methods, were made to observe the effect of ether on bacteria.

The methods used are described, and the result of the employment of each is summarized.

The following conclusions were drawn:

1. *Effective Strength of Alcohol for Destruction of Bacteria after Short Exposure.*—(*a*) *On a dry surface*:—The effective range of strengths of alcohol for the killing of non-sporing bacteria is between 90% and 50%. 95% and above are partially ineffective, 100% being markedly so. The lower surface tension of stronger alcoholic mixtures suggests that the upper limits of this effective range may be preferable to the lower, though against this must be considered the more pronounced fixing effect of strong alcohol, which may cause the coagulation of an exudate and the consequent protection of living organisms within the coagulum so formed (Bigger *et al.*, 1940). (*b*) *On the skin*:—Since the normal skin is more or less moist the effective range of alcohol for use upon it is somewhat different. 100% is commonly effective—at least on moister skins and under tropical conditions of temperature and humidity; while under similar conditions 60% to 65% may show a certain loss of efficiency. The washing of the skin before the application of alcohol might be expected to exert a similar effect unless subsequent drying were thorough. Further, the value of a low surface tension may well be of importance in increasing spread and penetration, for skin sterilization. It is therefore considered that, as a general recommendation under all climatic conditions, *80% of alcohol by volume* is probably most suitable for skin sterilization, though this will not be more effective than any other non-persistent agent for dealing with deep-lying resident flora.

2. *Bactericidal Effect of Ether.*—Ether is quite ineffective as a sterilizing agent for the skin, since its effect is very slight on staphylococci and streptococci when applied to a surface—though it is effective against certain bacillary forms, both Gram-positive and Gram-negative.

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REFERENCES

Bigger, J. W., *et al.* (1940). *British Medical Journal*, **1**, 79, 582.
Bonney, V., and Allen, H. S. (1944). Ibid., **2**, 210.
Cawston, W. C., and Colebrook, L. (1943). *Lancet*, **2**, 240 (correspondence).
Colebrook, L. (1941). *Bull. War Med.*, **2**, 73.
Cruickshank, J. C., *et al.* (1942). *British Medical Journal*, **2**, 182.
Garrod, L. P., and Keynes, G. L. (1937). Ibid., **2**, 1233.
Hailer, E., and Bockelberg, U. von (1939). *Arch. Hyg. Bakt.*, **122**, 20. (Reviewed in *Bull. Hyg.*, 640.)
Hatfield, C. A., and Lockwood, J. S. (1943). *Surgery*, **13**, 931.
Hayward, N. J., and Miles, A. A. (1943). *Lancet*, **2**, 116 (correspondence).
Lawrence, R. D. (1940). *British Medical Journal*, **1**, 150 (correspondence).
Lovell, D. L. (1945). *Surg. Gynec. Obstet.*, **80**, 174.
Martin, N. H. (1942). *British Medical Journal*, **2**, 245.
Miles, A. A., *et al.* (1944). *J. Path. Bact.*, **56**, 513.

The report for 1944 of the St. John Ophthalmic Hospital at Jerusalem has been issued from the Chancery of the Order, St. John's Gate, London, E.C. The hospital was founded in 1883, and during the year under review Field-Marshal Viscount Gort, High Commissioner of Palestine and Transjordan, paid a visit as a compliment to the work of the Order of St. John in Palestine. All the difficulties of perpetual changes of personnel have continued. So far as the decline in the number of patients is concerned, the Warden, Dr. Norman Manson, states that it is due to the impossibility of getting nurses and ward maids. This shortage is so acute in Palestine that several hospitals are almost closed. The improvement in the military situation has not been followed by any relief on the economic front; the cost of living is at least as high now as it was when Palestine was packed with troops. The clinical work has been carried out almost entirely by two surgeons and by two British Sisters with the support of a staff nurse seconded by the Director of Medical Services. The number of new cases seen during the year was 21,776, of whom 17,700 were Moslems, 3,270 Christians, and 806 Jews. The total number of patients suffering from acute conjunctivitis was 7,507, and 850 of these were complicated by corneal ulceration, which went on to perforation in 176.

ESTIMATION OF SERUM PROTEINS

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During recent years interest has been focused on the concentration of proteins in serum, as it is possible that, besides the gross reduction that is found in cases of famine oedema, a less severe reduction of the average concentration of protein in serum may occur among a population whose protein intake is low. If such less severe reductions are to be detected, the methods of estimating serum proteins must be such as will give average results which do not deviate from the true values, or values as estimated by some standard method, by at most more than 2%. It appears that the copper sulphate method recently introduced by Phillips, van Slyke, *et al.* (1944, 1945) is accurate enough if proper precautions are taken and the correct formula is used in calculating the protein concentration from the observed specific gravity.

Chibnall, Rees, and Williams (1943) have pointed out that considerable laxity has crept into the technique of estimation of nitrogen by the Kjeldahl method; their main criticism was that the times allowed for digestion of the proteins were not sufficient to ensure the conversion of all the nitrogen to ammonia. Our attention in this matter was aroused when we tried the copper sulphate method of measuring specific gravity. The relation between the specific gravity found (S) and number of grammes in 100 ml. of serum (P) is expressed by Equation 1:

$$P = K\ (S - A),$$

where K and A are constants. Moore and van Slyke (1930) found that concentrations of protein in serum of patients suffering from nephritis could be calculated from the specific gravity found by weighing. The appropriate values for K and A in Equation 1 for such sera were 343 and 1.007. However, the results obtained by Moore and van Slyke with 9 sera from normal persons did not fit this equation. In the first report (1944) of the copper sulphate method the values of K and A appropriate for this method were again given as 343 and 1.007. It was stated by workers in this country that concentrations found by the method, using this formula, agreed with those deduced from nitrogen estimations by the Kjeldahl method. We, however, found that if A was taken as 1.007 the value of K, when the method was used in sera from women before and shortly after delivery, was in the neighbourhood of 380; and in the later edition of their report on this method Phillips *et al.* (1945) give the value as 377 for use with normal sera, and 360 for use with pathological sera. The apparent discrepancies between the results of Kjeldahl estimations led us to reconsider the method that we were using.

Prof. Chibnall kindly had the total nitrogen of 15 sera, on which we also were working, estimated in his laboratory, using the reagents as described in the paper quoted and digesting for 17 hours. On the average, the results obtained by the method which we were then using were approximately 2% too low. We have assumed that the nitrogen found by Prof. Chibnall's technique was 100% of the true value. Table I shows the results obtained on a series of sera with different amounts of compounds of selenium and different times of digestion; in all these we used 0.2 ml. of serum, 0.17 ml. of 30% solution of copper sulphate, and 1 ml. of sulphuric acid; after 10 minutes' digestion 1 g. of sodium sulphate was added. If selenium was used as catalyst 0.1 ml. of sodium selenate solution (0.85 g. per 100 ml.) or an equivalent amount of selenium dioxide was added after cooling. The results are given in Table I.

To test the method most commonly used in clinical laboratories, 10 sera were digested with sulphuric acid and copper sulphate, but only 0.3 to 0.5 g. of sodium sulphate, until straw yellow; 2 or 3 drops of hydrogen peroxide were then added and the mixture boiled for a further 3 minutes. This method

returned 95.5% of the total. Even if the hydrogen peroxide was omitted the return was just under 95%.

As a routine we have digested with selenium dioxide for 3½ hours; in view of the above we can assume that the protein

TABLE I.—*Percentage of Nitrogen recovered in Kjeldahl Estimation (the amount obtained by 17 hours' digestion with selenate is taken as 100%)*

No. of Sera	Extra Catalyst Added	Time of Digestion	% Recovered
12	Selenium dioxide	3½ hrs.	99
21	Sodium selenate	50 mins.	99
17	Selenium dioxide	50 mins.	98·5
15	None	3½ hrs.	98
10	H_2O_2	3 mins.	95·5

concentrations calculated from our Kjeldahl nitrogens are certainly not too high, and probably on the average are about 1% too low. If A in Equation 1 is assumed to be 1.007 the value of K that fits our results is 384—giving Equation 2:

$$P = 384\ (S - 1.007).$$

However, we found that the specific gravities of the drops formed in copper sulphate solutions by the method described by Phillips *et al.* are less than those obtained by weighing in a pyknometer; while, if the drops are formed in the copper sulphate solution instead of being allowed to fall into it from above, the resulting specific gravity is approximately the same as that found by weighing. It is possible that this change in specific gravity is due to air that is entrapped during the fall. The value 1.007 for A was chosen because this is the specific gravity of protein-free ultrafiltrate from serum. If the difference in the specific gravities of drops formed inside and outside the copper sulphate solution is due to this entrapped air and not to a change in the serum protein it is reasonable to change the value of A and to retain a value of K which is appropriate for true specific gravities. We have found that Equation 3,

$$P = 366\ (S - 1.007),$$

fits our results obtained by weighing in a pyknometer. If this value of K is used the value of A obtained for normal sera is 1.0061. For convenience of calculation we propose Equation 4,

$$P = 364\ (S - 1.006).$$

The difference in the calculated results introduced by this change in K and A is negligible. A variety of equations, with different values of K and A, can be propounded that will fit the values of the apparent specific gravity as found in the copper sulphate method and of protein concentration found in normal sera, all containing between 6.5 and 7.8 g. of protein per 100 ml. To determine the two constants we need sera containing less or more protein. The obvious sera to use would be those of patients with nephritis and oedema. However, the figure given by Moore and van Slyke (1930) suggests that the constants suitable for such cases differ from those suitable for normal sera. As this method will probably be used to estimate proteins in sera of persons suffering from malnutrition, a form of the equation suitable for such sera will be appropriate. In the 6 sera with the lowest serum proteins obtained from returned prisoners of war we arrived at the results shown in Table II.

TABLE II.—*Protein Concentration in Sera of Returned Prisoners of War, calculated from the Specific Gravity by Equations 4 and 2 and from the Nitrogen, calculated by Kjeldahl's Method*

Case	Kjeldahl	Equation 4	Equation 2
3	6·14	6·08	6·03
15	5·27	5·17	5·07
11	5·97	5·93	5·88
18	5·51	5·39	5·30
5	5·88	6·01	5·95
16	6·02	6·19	6·14

In 4 out of the 6 the concentrations calculated by Equation 4 agree better with the Kjeldahl results than those calculated by Equation 2.

With 101 sera from "normals" (blood donors, laboratory technicians, and students), normal pregnant women, normal women after delivery, and returned prisoners of war, all of whom had lost much in body weight, we obtained the results shown in Table III.

TABLE III.—*Differences between Protein Concentration calculated from the Specific Gravity by Equation 4 and from Nitrogen Estimation*

Per cent.	Normals	Returned Prisoners of War	Ante-natal	Post-natal
+ 5·0 to + 5·9	0	0	0	1
+ 4·0 to + 4·9	1	0	0	2
+ 3·0 to + 3·9	0	2	1	6
+ 2·0 to + 2·9	0	5	1	4
+ 1·0 to + 1·9	3	2	4	3
− 0·9 to + 0·9	10	7	11	12
− 1·0 to − 1·9	4	2	5	2
− 2·0 to − 2·9	1	5	5	0
− 3·0 to − 3·9	1	1	4	1
− 4·0 to − 4·9	0	1	0	0
− 5·0 to − 5·9	0	0	1	0
Total number	20	25	32	31
Mean difference %	−0·2	−0·1	−1·0	+1·4
K calculated statistically	354	359	327	345
A " "	1·0054	1·0057	1·0037	1·0052
K calculated for A = 1·006	365	364	368	359

It should be noted that Equation 4 is based primarily on the assumption with regard to the value of A. Statistical calculation of the equation that fitted the results would give lower values for A in Equation 1.

Phillips *et al.* (1945) in their second description of the method state that the equation suitable for pathological sera for A in Equation 1.

$$P = 360\ (S - 1.007).$$

We have, however, found that Equations 2 and 4 fit the pathological sera that we have examined. Phillips *et al.* also recommend that Equation 5 should be used generally for all sera. But this would result in a discrepancy of about 6% between estimations made by this method and those based on accurate estimations of the protein nitrogen.

Specific gravity methods have been criticized in the past because they have given results that disagreed with those derived from Kjeldahl estimations. It appears, however, that some of the Kjeldahl methods in use are liable to errors of at least 10%; the grosser discrepancies may be due to faults in the Kjeldahl methods used. The copper sulphate method is extremely simple and convenient. If a stock solution accurately made up or even a series of standard solutions were issued from a centre, gross discrepancies between the results of estimations of serum proteins would be avoided.

The drops should be allowed to fall into the fluid, as described by Phillips *et al.*; if formed in the fluid, a separate dropping pipette must be used for each drop and much of the convenience of the method is lost. In an established laboratory a gradient method as described by Jacobsen and Linderström-Lang (1940–1) may be quicker and more convenient; we propose to publish a paper on this method shortly. Both these methods have the advantage over other specific gravity methods that changes of temperature have little effect.

The specific gravity which we have considered is the ratio of the weight of a given volume of serum to the weight of an equal volume of water, both at 24° C. The difference in the specific gravity observed, if the measurement is made at a different temperature, is only about 0.00001 per degree, as the coefficient of expansion of serum differs little from those of the copper sulphate solutions. If, therefore, the specific gravity is measured at a temperature as low as 14° or as high as 34°, instead of 24°, the error in the calculated concentration of protein would amount to only about 0.04 g. per 100 ml.

As Phillips *et al.* point out, it is essential that, in preparing the standard solutions, the stock solution and water used for dilution should be at the same temperature. If this precaution is observed the specific gravities of a standard solution at 24° (that is, the ratio of the weight of a given volume of solution at 24° to that of the same volume of water also at 24°) will not vary by more than ±0.0001 if the dilution is done at any temperature between 10° and 40°. The stock solution should be prepared at 24°; but an error of not more than ±0.0001 in the specific gravities of the standards will be introduced if the solution is made up at any temperature between 14° and 34°. The volume of the drop of serum should be about 0.02 ml. The

height from which it falls should be such that it forms a ring when it enters the fluid. If cups or umbrellas are formed the apparent specific gravities will be higher; if the pipette is not steady fluffy rings will be formed and the apparent specific gravity found will be less.

Summary

The copper sulphate method for measuring specific gravity of serum gives satisfactory and consistent estimates of the protein concentration.

The apparent specific gravities found by this method are less than the true specific gravity.

The equation recommended for calculating the protein concentration from the apparent specific gravity is $P = 364 (S - 1.006)$.

For satisfactory and consistent measurements of serum protein by the Kjeldahl method, selenium dioxide should be used as a catalyst and digestion should be continued for at least one hour, and preferably for 3½ hours.

We wish to express our thanks to Prof. Chibnall for his advice and assistance.

REFERENCES

Chibnall, A. C., Rees, M. W., and Williams, E. F. (1943). *Biochem. J.*, **37**, 354.
Jacobsen, C. F., and Linderström-Lang, K. (1940–1). *Acta physiol. scand.*, **1**, 149.
Moore, N. S., and van Slyke, D. D. (1930). *J. clin. Invest.*, **8**, 337.
Phillips, R. A., van Slyke, D. D., Emerson, K., Hamilton, P. B., and Archibald, R. M. (1944 and 1945). *Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma*, Josiah Macy, jun., Foundation, New York.

FOUR CASES OF TYPHUS FEVER IN GREAT BRITAIN

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Typhus fever is rare in Great Britain. From a review of the recent literature on its incidence it would appear that only one case occurring in 1944 has been recorded (Agnew and Kyles, 1944). As several cases of typhus fever are now under treatment in this country, it may prove of interest to report a small series of four patients who developed this disease while in hospital in Bradford. Three of these cases had been under constant medical supervision before the development of the disease, and it has thus been possible to follow the course of their illness from the day of onset.

The four patients referred to in the following report had been released from the same prisoner-of-war camp in Germany, in which, so far as can be ascertained, typhus fever was endemic. They were all disinfested by the routine procedure of insufflation of their clothing by dichlor-diphenyltrichlorethane (D.D.T.) powder on April 26, 1945. They arrived in England two days later and were immediately transferred to hospital, where on admission they were found to be free from lice.

Case I

A man aged 25. His general condition on admission was fairly satisfactory, his weight then being about 14 lb. below his normal. On May 3—i.e., seven days after disinfestation—there was an unexplained rise of temperature to 102° F., with a pulse rate of 124. Prodromal symptoms were entirely absent, apart from a feeling of "fatigue," which persisted throughout the illness. The pyrexia and tachycardia continued for 10 days and then fell by slow lysis over a period of four days. The total duration of the febrile period was 13 days, and while during this time the patient developed a fairly well marked degree of toxaemia, this at no time gave rise to any anxiety. His mental condition remained fairly normal throughout the illness, and although towards the end of the first and beginning of the second week there was definite mental confusion, there was neither violent delirium nor restlessness. Conjunctival suffusion was present at the start of his illness. The rash, which was reported to be macular in character, developed on the fourth day and showed the typical distribution over the trunk and limbs only. When seen by one of us eight days later it had entirely disappeared. There was no splenomegaly. On the 14th day of the disease there was clinical evidence of meningism, but lumbar puncture revealed a normal cerebrospinal fluid. The following pathological investigations were made:

5th day of disease:
Widal reaction = Positive agglutination to 1 : 50 against *B. typhosus*
12th day of disease:
Weil-Felix reaction: agglutination against OX 19 to 1 : 250
14th day of disease:
Weil-Felix reaction: agglutination against OX 19 to 1 : 1000

The patient made an uninterrupted recovery.

Case II

A man aged 44. The general condition of this patient remained satisfactory until May 5—i.e., 9 days after disinfestation—when there was a rise in his temperature and pulse rate to 99° F. and 100 respectively. The next day his temperature had risen to 104° F. At this time the patient had a septic right thumb, which required incision and drainage. From this, the 4th day of his illness, his general condition deteriorated steadily, so that by the 10th day the typhoid state was well established. There was a low muttering delirium, and signs of cardiovascular failure soon became apparent. When seen by one of us on the 12th day he was *in extremis*, and died within 24 hours. On the 6th day a subcuticular mottling appeared over the trunk, together with a petechial rash, which was profuse on both trunk and limbs. On the 10th day the spleen was reported to be slightly enlarged. Conjunctival injection was marked.

Pathological reports included the following relevant findings:—On the 11th day of disease: White blood count, 16,000 per c.mm.—polymorphs, 64%; lymphocytes, 28%; Türck cells, 2%; mononuclear leucocytes, 6%; blood urea, 86 mg. per 100 c.cm. Urine: Albumin ++; a few granular and epithelial casts present. Weil-Felix reaction: Agglutination against OX 19 to 1: 10,000; against OX 2 to 1: 50.

At the necropsy all the organs showed evidence of passive congestion. The spleen was normal in size, with soft diffluent pulp. There was a diffuse rash over the body, merging with post-mortem lividity. Discrete small macules were present on the trunk, and were profuse on the legs and inner aspects of the arms. A few petechial haemorrhages were also present.

Report on the skin section: In the skin macule the small vessels showed marked congestion, with collections of inflammatory cells (mainly lymphocytes) in and around the adventitia.

Case III

A man aged 25. This patient was discharged home on leave four days after his arrival in England and was readmitted to hospital 12 days later. He stated that for the preceding six days he had been suffering from vomiting and diarrhoea with severe headache and backache, the illness beginning with a shivering attack The illness commenced 11 days after disinfestation, assuming that the onset dated from this shivering attack. On admission his temperature was 100.6° F. and pulse rate 94. The pyrexia which followed was of an irregular character and reached 104.8° F. on the 9th day of the illness, and there was a tachycardia of 150 on the 11th day. The febrile period continued for 13 days and then fell by slow lysis over 4 days. The rash appeared on the 9th day, and consisted of petechiae, mainly present on the abdomen and legs, but this distribution soon involved the whole trunk and limbs. At this time there was a very well marked conjunctival suffusion. An enlarged spleen was easily palpated and was found to extend two fingerbreadths below the left costal margin. During his illness there was a mild toxaemia, accompanied by lethargy and a fine tremor of hands and feet. There was also some mental confusion, most marked at night, but no undue restlessness.

The pathological findings were:—On the 9th day of disease: Blood urea, 90 mg. per 100 c.cm. Urine: Hyaline and occasional granular casts present. Weil-Felix reaction: Agglutination against OX 19 to 1: 250. On the 16th day of disease the Weil-Felix reaction showed agglutination against OX 19 to 1: 1000. The patient had a rapid convalescence.

Case IV

A man aged 29. He was a contact of Case I, being a patient on the opposite side of the ward, but as he was ambulant during the early part of the latter's illness a closer contact may have occurred. On the 27th day after disinfestation the patient complained of nausea, fairly severe frontal and occipital headache, and malaise. His temperature and pulse rate in 12 hours rose from normal to 103.2° F. and 96. This rise was followed by a short febrile period of only 5 days, when the temperature was of an irregular character, as in the other three cases. Apart from a mild conjunctival injection, there were no other clinical signs during this man's illness.

Pathological Report.—2nd day of disease: Weil-Felix reaction—positive agglutination against OX 19 to 1: 50 and against OX K to 1: 25; 6th day of disease: Positive against OX 19 to 1: 1000. The patient's general condition remained excellent throughout, and his symptoms rapidly disappeared when his temperature had subsided.