and protein hydrolysates had been prepared on a big scale, and controlled experiments were conducted in Holland under the direction of Prof. Beattie: they could be given orally, by nasal tube, or intravenously. In Belsen they were used on a small scale; but they were most nauseating by mouth. Patients had to be persuaded to take their drinks, and extensive nasal feeding was impracticable; their superiority over the simpler diets has not been established. *Oedema:*—This usually disappeared with a good diet. If it was very severe salyrgan intravenously was effective and seemed harmless. *Diarrhoea:*—As the cause was not clear, rational treatment was out of the question. The multiplicity of treatments found to be effective suggests that none was specific. *Intravenous Plasma:*—At Sandbostel Camp Capt. A. R. Blower carried out a most extensive experiment with plasma; he used 1,196 pints—an average of 5.1 pints per patient. His conclusions (personal communication) were that it had no effect on diarrhoea; it gave good results in severe wasting; and in oedema the results were inconclusive.

Penicillin

We had ample supplies of penicillin, and every opportunity was seized of studying its use in medical as opposed to surgical conditions. Owing to its non-toxicity and its action on a wide range of organisms, it would replace sulphonamides were its method of administration by repeated injection not so impracticable except in hospitals: the sulphonamides remained our sheet anchor in acute infections except from resistant organisms. Penicillin's greatest value to a fighting army was in reducing the time spent in hospital for simple ailments. The Adviser in Venereology (Lieut.-Col. D. J. Campbell) was able to reduce the time of treatment for gonorrhoea to 24 hours, and for syphilis to a little over a week if treated early in the primary stage. In simple pyodermias the Adviser in Dermatology (Lieut.-Col. F. F. Hellier) exploited its use to the full, even in minor medical units with the field force, and saved countless man-days. In ulcerative gingivitis its local application seemed to be specific, and the penicillin tablet should be the simple answer to this common ailment.

In the serious infections, where our object was to save life and not man-days in hospital, it proved invaluable in staphylococcal septicaemia and staphylococcal pneumonia. In pneumococcal pneumonia it was not better than sulphonamides. In meningococcal meningitis it proved to be curative if given intrathecally; but the many lumbar punctures needed make its use objectionable, and there is the danger of introducing resistant organisms into the theca. In Weil's disease it is of value in some strains if given very early. In acute diphtheria it is a useful adjunct to adequate antitoxin, and may clear the throat of bacilli more quickly than the average time taken.

Conclusion

I fear I have done scant justice to the team of British and Canadian physicians with whom it was my privilege to be associated; this is their work, and I have made myself their mouthpiece. They, working for the most part under improvised, difficult, and sometimes dangerous conditions, maintained a high standard of medicine, and a standard of acute medicine which I know I shall not again see. I regret that individual acknowledgment is possible only in a few instances.

The Canadian side, with its happy blend of British and American medicine, was always an inspiration, and I would thank Col. Murray Baird, R.C.A.M.C., their Consulting Physician, for his constant help. Finally, my thanks are due to the Director of Medical Services, 21 Army Group, Major-Gen. E. Phillips, for permission to publish this article, but more especially for his consistent support of, and constant sympathy with, the clinical side.

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E. C. Ross Couper (*Arch. Dis. Childh.*, 1945, **20**, 117), from a study of twenty cases of various infections in infants, concludes that penicillin has a useful part to play in parenteral infection in infancy, but much further investigation is needed.

ESTIMATION OF SERUM PROTEINS BY THE LINDERSTRÖM-LANG GRADIENT

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The gradient method described by Jacobsen and Linderström-Lang (1940–1) affords a very quick and convenient means of measuring the specific gravity of serum and hence of estimating the concentration of protein in serum, as only the drop is needed. In this method a light and a heavy mixture of kerosene and bromobenzene are prepared. The lighter mixture is superposed on the heavier in a glass cylinder and the column of fluid is stirred. A zone is formed in which the specific gravity decreases with the height. The specific gravity of serum introduced into the column is estimated by comparison with the position taken up by drops of salt solution of known specific gravity. The apparent specific gravities found by this method differ from the true specific gravities, although the difference is less than that found in the copper sulphate method (Hoch and Marrack, 1945). This difference is reduced if chlorobenzene is used instead of bromobenzene.

Method

As the journals in which the preparation of this gradient is described are not available in all libraries, details of this preparation, with slight modification, are here given.

The Gradient.—Two mixtures of chlorobenzene (spec. grav. $S^{20}_{20}=1.109$) or bromobenzene (spec. grav. $S^{20}_{15}=1.499$) with kerosene (spec. grav. approximately 0.80) are prepared so that their specific gravities $S^{t^\circ}_{t^\circ}$ (t^0 = room temperature) are approximately equally spaced about the midpoint of the range required. The specific gravities of these mixtures should be approximately 0.04 above and 0.04 below that of the midpoint of the range of specific gravities required. For example, two mixtures prepared by mixing chlorobenzene and kerosene in the ratio of approximately 7.5 and 0.69 by volume had specific gravities ($S^{24^\circ}_{4^\circ}$) 1.064 and 0.982. At the midpoint of the gradient prepared with these mixtures the specific gravity $S^{24^\circ}_{4^\circ}$ was 1.023 and $S^{24^\circ}_{24^\circ}$ was 1.0257, corresponding to a concentration of protein in serum of 6.75 g. per 100 ml.

About 100 ml. of the heavier liquid is placed in a wide graduated cylinder (e.g., a 500-ml. measuring-cylinder which has been cut off at the 250-ml. mark) and the same amount of the lighter liquid is superposed by letting it run in slowly down a glass rod which touches the wall of the cylinder near the surface of the liquid. The two liquids are then mixed by moving a glass rod, bent at the lower end, up and down in the column with continued rotation until streaks (schlieren) appear at the bottom and top of the column. The gradient should then be tested with two drops of standard solutions, specific gravity 1.020 and 1.030. The distance between them should be 4 to 5 cm. The apparent specific gravity of a drop can be read with an accuracy of at least ± 0.0002.

As emphasized by Linderström-Lang (1938), saturation with water is essential. After the gradient is made up a set of 10 drops of the standard solutions should be left suspended in the column for at least a day before it is used. The cylinder should be kept in a water jacket in order to prevent rapid changes of temperature.

Standard Solutions.—The series of copper sulphate solutions with specific gravities differing by 0.001 prepared from a stock solution ($S^{24}_{24}=1.1000$; 159.6 g. in 1,000 ml. at 24° C.), as described by Phillips, van Slyke, *et al.* (1944, 1945), can be used. A series of drops covering the range of specific gravities anticipated is introduced into the column by the method described for serum. Fresh drops of the standard solution should be introduced if the old ones have been disturbed on removing a batch of drops of serum.

Procedure.—With a narrow pipette, attached to a rubber bulb, take up between 5 and 15 c.mm. of serum. Bring the point of the pipette about 3 mm. below the surface of the fluid in the cylinder. Blow the serum out gently so that it does not leave the point of the pipette until the pipette is withdrawn from the surface of the fluid. A slight positive pressure should be maintained throughout. Drops of 5 to 15 c.mm. are recommended instead of 1 c.mm. as used by Jacobsen and Linderström-Lang (1940–1), in order to increase the rate of fall and, in the case of serum, to delay solution of chlorobenzene or bromobenzene. Readings should be taken at the centres

of the drops, with precaution to avoid errors due to parallax. Every measurement is referred to the position of the nearest drops of standard solution by means of linear interpolation at the time of reading. Although the distances between two pairs of standard drops that differ in specific gravity by equal amounts may be found to be equal, it is possible that there may be occasional irregularities in the gradient; but the error in the estimate of specific gravity of the serum is not likely to exceed 0.0005. The level of the drops should be read 10 minutes after their introduction. This time was chosen because (*a*) it exceeds the time (2 minutes) required by drops of the standard solution to reach equilibrium to within a specific gravity of 0.0001, and (*b*) in this time the increase of specific gravity of the drop of serum, due to solution of chlorobenzene or bromobenzene, becomes so slow that no appreciable change in its level occurs while the reading is being taken. Estimations can be made on several sera before the drops are removed.

Removal of Drops.—Moisten a piece of filter-paper and wrap it round the lower end of a long thin glass rod. Bring this in contact with the drops, which will adhere to the paper. After removal of the drops the gradient should not be used again for 30 minutes.

Changes with Time.—The mixture gradually becomes uniform throughout; changes in two weeks may be appreciable.

Recovery of Reagents.—The chlorobenzene or bromobenzene can be separated from the kerosene, in a state fit for use in making a fresh gradient, by distillation at normal pressure. Unless the distillation is carried out under reduced pressure, part of the kerosene must be replaced by fresh material.

TABLE I.—*Comparison of Specific Gravities Measured by Direct Weighing and by the Chlorobenzene Gradient*

Case	Direct Weighing $S\frac{24°}{24°}$*	Gradient $S\frac{24°}{24°}$†	Difference: Gradient/Direct Weighing $S\frac{24°}{24°}$	Equivalent (g. protein/100 ml.)
1	1·0262$_5$	1·0264$_0$	0·0001$_5$	0·05
2	1·0266$_5$	1·0269$_0$	0·0002$_5$	0·09
3	1·0270$_5$	1·0273$_5$	0·0003$_0$	0·11
4	1·0270$_0$	1·0271$_5$	0·0001$_5$	0·05
5	1·0247$_5$	1·0249$_0$	0·0001$_5$	0·05
6	1·0245$_0$	1·0248$_0$	0·0003$_0$	0·11
7	1·0260$_0$	1·0261$_0$	0·0001$_0$	0·04
8	1·0263$_0$	1·0264$_5$	0·0001$_5$	0·05
9	1·0261$_5$	1·0264$_0$	0·0002$_5$	0·09
10	1·0250$_0$	1·0252$_5$	0·0002$_5$	0·09
11	1·0246$_0$	1·0251$_0$	0·0005$_0$‡	0·18
12	1·0255$_5$	1·0257$_5$	0·0002$_0$	0·07
13	1·0255$_5$	1·0259$_0$	0·0003$_5$	0·13
14	1·0244$_5$	1·0246$_5$	0·0002$_0$	0·07
15	1·0253$_5$	1·0256$_5$	0·0003$_0$	0·11
16	1·0248$_0$	1·0247$_5$	0·0000$_5$	0·00
17	1·0237$_5$	1·0238$_5$	0·0001$_0$	0·04
18	1·0266$_5$	1·0268$_5$	0·0002$_0$	0·07
19	1·0262$_5$	1·0263$_5$	0·0001$_0$	0·04
20	1·0275$_0$	1·0277$_0$	0·0002$_0$	0·07
Post-natal:				
81	1·0243$_5$	1·0245$_0$	0·0001$_5$	0·05
82	1·0267$_5$	1·0267$_5$	0·0000$_0$	0·00
Ante-natal:				
83	1·0254$_0$	1·0255$_5$	0·0001$_5$	0·05
84	1·0247$_5$	1·0249$_5$	0·0002$_0$	0·07
94	1·0255$_5$	1·0256$_0$	0·0000$_5$	0·02
95	1·0240$_5$	1·0240$_0$	−0·0000$_5$	−0·02
96	1·0266$_0$	1·0267$_0$	0·0001$_0$	0·04
97	1·0247$_0$	1·0249$_0$	0·0002$_0$	0·07
98	1·0257$_5$	1·0258$_0$	0·0000$_5$	0·02
		Average difference:	+0·0001	0·06

* Corrected to 24° C. by applying −0·00005 per ° C.
† Measured between 19° and 22·5° C., uncorrected for temperature; the extreme correction would not exceed 0·00005.
‡ This large difference was probably caused by the error in the determination by direct weighing.

Effect of Temperature

One of the advantages of the copper sulphate and gradient methods, as compared with direct weighing and the falling-drop method, is that serum is compared with aqueous solutions whose coefficients of expansion differ little from those of sera. If the slight effect of temperature on the difference of solubilities of chlorobenzene in serum and copper sulphate solution is disregarded, the effect of temperature is the same as that in the copper sulphate method: the error in the calculated concentration of protein due to change of temperature up to 34° C. or down to 14° C. will not amount to more than 0.04 g. per 100 ml.

TABLE II.—*Comparison of Apparent Specific Gravities ($S\frac{24}{24}$) found by the Chlorobenzene and Bromobenzene Gradients*

Case	Readings after 10 Minutes			Readings after 60 Minutes		
	Chloro-benzene	Bromo-benzene	Difference Br/Cl	Chloro-benzene	Bromo-benzene	Difference Br/Cl
Malnutrition:						
21 ..	1·0286$_5$	1·0289	0·0002$_5$	1·0287$_5$	1·0293$_5$	0·0006
22 ..	1·0263	1·0266$_5$	0·0003$_5$	1·0264$_5$	1·0269$_5$	0·0005
23 ..	1·0244$_5$	1·0247$_5$	0·0003	1·0247	1·0252$_5$	0·0005$_5$
24 ..	1·0257	1·0261	0·0004	1·0260	1·0268	0·0008
25 ..	1·0237$_5$	1·0241	0·0003$_5$	1·0240	1·0247	0·0007
26 ..	1·0264	1·0265$_5$	0·0001$_5$	1·0265$_5$	1·0271	0·0005$_5$
Ante-natals:						
69 ..	1·0255	1·0257$_5$	0·0002$_5$	1·0257	1·0261$_5$	0·0004$_5$
70 ..	1·0264	1·0265	0·0001	1·0265$_5$	1·0267$_5$	0·0002
73 ..	1·0249	1·0253	0·0004	1·0251$_5$	1·0258$_5$	0·0007
74 ..	1·0270	1·0272$_5$	0·0002$_5$	1·0271	1·0277	0·0006
75 ..	1·0264	1·0271	0·0007	1·0266	1·0272	0·0006
78 ..	1·0289$_5$	1·0291$_5$	0·0002	1·0293	1·0296	0·0003
79 ..	1·0274$_5$	1·0278	0·0003$_5$	1·0276	1·0281$_5$	0·0005$_5$
Post-natals:						
71 ..	1·0257	1·0261	0·0004	1·0260	1·0262$_5$	0·0002$_5$
72 ..	1·0266$_5$	1·0271	0·0004$_5$	1·0270	1·0274	0·0004
76 ..	1·0273$_5$	1·0277	0·0003$_5$	1·0277	1·0282$_5$	0·0005$_5$
77 ..	1·0263	1·0266	0·0003	1·0266	1·0270	0·0004
Cord blood:						
31 ..	1·0289	1·0291	0·0002	1·0289	1·0292	0·0003
32 ..	1·0257	1·0261	0·0004	1·0259	1·0263	0·0004
33 ..	1·0253$_5$	1·0255$_5$	0·0002	1·0253$_5$	1·0255$_5$	0·0002
Diabetes:						
Bl ..	1·0245	1·0252	0·0007	1·0246$_5$	1·0257	0·0010$_5$
B ..	1·0264	1·0267	0·0003	1·0266	1·0269$_5$	0·0003$_5$
A ..	1·0274	1·0277$_5$	0·0003$_5$	1·0276	1·0281$_5$	0·0005$_5$
	Average difference .. 0·00035 Average protein equivalent, g./100 ml. .. 0·13			Average difference .. 0·00052 Average protein equivalent, g./100 ml. .. 0·19		

TABLE III.—*Number of Cases in which the Difference % between the Concentration of Protein calculated from the Observed Specific Gravity by Equations 2 and 3 and that calculated from the Nitrogen Content lay between Certain Limits. Values of K and A in Equation 1, calculated statistically*

Difference % of Values Calculated from Specific Gravity and Kjeldahl's	Chlorobenzene Gradient				Direct Weighing		
	Normals	Malnutrition	Ante-natals	Post-natals	Normals	Ante-natals	Post-natals
+5 to +5·9 ..	—	—	—	1	—	—	—
+4 to +4·9 ..	—	—	—	0	—	—	—
+3 to +3·9 ..	—	4	—	4	1	—	2
+2 to +2·9 ..	—	3	1	4	0	—	0
+1 to +1·9 ..	2	2	3	4	3	—	5
+0·9 to −0·9 ..	19	10	5	3	13	2	0
−1 to −1·9 ..	4	4	4	3	2	2	1
−2 to −2·9 ..	—	1	2	—	1	5	—
−3 to −3·9 ..	—	1	5	—	—	3	—
−4 to −4·9 ..	—	—	—	—	—	1	—
Greatest difference, negative side ..	−1·5	−3·4	−3·8	−1·9	−2·1	−4·1	−1·7
,, ,, positive side ..	+1·9	+3·8	+2·6	+5·0	+3·0	−0·2	+3·7
Total number ..	25	25	20	19	20	13	8
Average difference in % of Kjeldahl ..	−0·16	+0·54	−1·1	−1·5	−0·05	2·3	−1·6
Range of protein content (Kjeldahl) ..	6·16–7·44	5·27–7·81	6·27–7·99	6·24–7·54	6·16–7·39	6·22–7·38	6·24–8·48
K calculated statistically ..	359·2	353·3	364·6	348·0	358·5	—	—
A calculated statistically ..	1·0069	1·0067	1·0070	1·0066	1·0066	—	—
K calculated for A = 1·007 ..	361·6	359·3	364·6	355·6	365·3	374	359

Effect of Solution of the Halogen-benzene in Serum

The apparent specific gravity of serum in the chlorobenzene gradient is higher than that found by weighing in a pyknometer (Table I), and the apparent specific gravity in the bromobenzene is higher than in the chlorobenzene gradient (Table II). These differences are apparently due to preferential solubility of the halogen-benzene in the serum. The solubility of bromobenzene in water is 0.045 g. per 100 ml.; the increase of the specific gravity of a drop of water owing to saturation with bromobenzene is about 0.0002. The halogen-benzenes are presumably much more soluble in serum than in water owing to the lipids in the serum. As the difference between the specific gravities of bromobenzene and serum is much greater than that between those of chlorobenzene and serum, a small excess in the amount dissolved in serum over that dissolved in the standard drops has a considerably greater effect in the case of the bromine compound. The specific gravities of the drops increase as the serum becomes saturated with the halogen compound; hence the differences between the specific gravities found in the bromobenzene and the chlorobenzene gradients are greater at 60 minutes after the drops are introduced than at 10 minutes. After the first 10 minutes the increase of specific gravity in the chlorobenzene gradient amounts to only 0.0002 in 50 minutes.

The difference in the solubility of the two compounds is illustrated by the behaviour of drops of different sizes in the two gradients. Within a short interval two pairs of drops of size about 50 c.mm. and 1 c.mm. were dropped into a chlorobenzene and a bromobenzene gradient. During the first 10 minutes in the bromobenzene gradient the small drop sank much below the level of the large drop; while in the chlorobenzene gradient the two drops were nearly level. After 6 hours the small and large drops were exactly level in both gradients.

Reproducibility and Accuracy

The readings obtained when two drops of the same serum were added to the same gradient in immediate succession were always identical. The specific gravities found using different gradients, or the same gradient at different temperatures, always agreed within 0.0004 and usually within 0.0002.

The concentrations of protein in serum calculated ($N \times 6.25$) from estimation of nitrogen by the Kjeldahl method were taken as the true concentrations. The values of K and A in Equation 1

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

(where P is the concentration of protein in g. per 100 ml. and S the observed specific gravity), calculated statistically, are given in Table III. For convenience in calculation, in practice, the equations adopted were: Equation 2 for specific gravity found by direct weighing:

$$P = 365\ (S - 1.007)$$

and Equation 3 for apparent specific gravity found by the chlorobenzene gradient:

$$P = 361\ (S - 1.007).$$

The readings can also be made with fair accuracy 40 to 60 seconds after the introduction of the drop. If this is done the value of A should be 1.0069.

Using these equations, the agreement between the results obtained by direct weighing or the chlorobenzene gradient on the one hand and by the Kjeldahl method on the other is remarkable in the normal sera and satisfactory in others. As with the copper sulphate method, the average values found by specific gravity methods in sera from women during pregnancy and after delivery tended to differ in opposite directions from those found by the Kjeldahl method. This difference, which may be due to the higher concentration of lipids in the serum during pregnancy, is being studied further.

The "normal" sera were obtained from blood donors at the end of bleeding, after lying down for not less than 10 minutes; the concentrations of proteins found may therefore be abnormally low. The other subjects were bled with precaution to avoid stasis.

Since Table III was completed we have received a serum from a patient with extreme hypoproteinaemia, probably due to faulty nutrition. The concentrations of protein found were: Kjeldahl, 2.54 g./100 ml.; copper sulphate method (Equation 4, $P = 364\ (S - 1.006)$, Hoch and Marrack, 1945), 2.55 g./100 ml.; chlorobenzene gradient, 2.64 g./100 ml. The equations proposed therefore give satisfactory results when the concentration of protein in serum is very low.

[As this paper was going to press we received an article by Lowry and Hunter (*J. biol. Chem.*, 1945, **159**, 465) which gives a full description of the gradient method, using bromobenzene, with a discussion of its accuracy.]

Summary

The gradient method of Linderström-Lang is a convenient and rapid means of estimating the concentration of protein in serum.

The apparent specific gravities found by this method are higher than the true specific gravities. This difference is attributed to preferential solution of the halogen-benzene in the serum; it is less if chlorobenzene is used in the place of bromobenzene. Within moderate limits the effect of temperature is negligible.

Using chlorobenzene, satisfactory and consistent measurements of the concentration of protein are obtained.

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LIVER FUNCTION IN INFECTIVE HEPATITIS GAUGED BY HIPPURIC ACID SYNTHESIS TESTS*

BY

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Up till recently the hippuric acid synthesis test has been employed mainly as an indication of liver damage in isolated instances, particularly in the differential diagnosis of jaundice. In the present work the test has been used serially in an attempt to follow the course of liver damage in cases of infective hepatitis. Although there are many complications, both theoretical and practical, in the interpretation and analysis of the results of this test, it was decided that it was probably the most reliable of known guides to liver function, particularly in the presence of jaundice. Moreover, one of the more serious objections to the test—the wide "normal" range of figures obtained in healthy persons—does not apply when following liver function by serial tests in the same individuals.

Methods and Technique

Cases of infective hepatitis were admitted to hospital usually within three days of the appearance of jaundice. In most instances the serum bilirubin and hippuric acid synthesis were first estimated the next morning. The serum bilirubin was subsequently estimated (Malloy and Evelyn, 1937) twice weekly until the peak value was passed, and thereafter once a week until discharge; estimations were not always done on the same day as the hippuric acid test, and many of the values on which the accompanying Table is based have been obtained by interpolation between the nearest figures before and after the date of the test. The second hippuric acid test was performed usually just under a week after admission, and a third and final test was done a week to 10 days before discharge. The method used was the intravenous modification of Quick (1939), in which 1.77 g. of sodium benzoate was injected intravenously and the urine collected after one hour. The amount of hippuric acid thus excreted was estimated by the technique recommended by Weichselbaum and Probstein (1938), and expressed in terms of the quantity of sodium benzoate detoxicated. The present series includes 81 cases, 46% of which were admitted with rising serum bilirubin. Patients who developed relapses have been excluded.

Results

The results are recorded in the accompanying Table, which is so arranged that a direct comparison can be made between the hippuric acid results and the serum bilirubin concentration.

* A report to the Medical Research Council.