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 pycnometer (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 Probst (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.

Cases have been divided into two main groups (A and B) according to whether they were admitted with rising or with falling serum bilirubin. These groups have been further subdivided arbitrarily into four roughly equal subgroups (1, 2, 3, and 4) according to the maximum recorded serum bilirubin values. The main points can be summarized thus:

(i) The hippuric acid synthesis (H.A.) appears to reflect the severity of the attack and the stage of the disease rather than to be directly correlated with the serum bilirubin concentration. For instance, there is a significant difference (Difference/Standard Error=2.84) between the initial H.A. values in Subgroups 1 and 2, indicating a correlation between H.A. synthesis and severity of attack fairly early in the disease. There is also a significant difference (D./S.E.=2.33) between the initial H.A. values in Subgroups 1 and 3, although in this case the bilirubin concentrations are roughly equal; in Subgroup 1, however, the serum bilirubin is rising, and in Subgroup 3 falling; it is not surprising, therefore, that liver function in Subgroup 3 is better than in Subgroup 1. The difference applies only to the more severe cases, and is not apparent in Subgroups 2 and 4.

(ii) There is a considerable increase in H.A. values during the first 6 days in all groups. Even in Subgroup 1 the mean rise (0.126 ± 0.0125) is appreciable. The rise in Group B (0.325) is well over double the rise in Group A (0.139), consisting of cases admitted earlier in the disease, and the difference between the improvements in the two groups is highly significant (D./S.E.=3.91). It is of interest that this improvement in H.A. synthesis during the first week after admission occurs despite an increase in serum bilirubin. In Subgroup 1 it can be seen that there is a large mean rise of nearly 3 mg. per 100 ml. in serum bilirubin over the same period as the improvement in H.A. synthesis.

(iii) There is little difference between the groups in the mean H.A. synthesis before discharge. With the exception of Subgroup 1 this final test was performed, on an average, at the same interval after admission in all groups. Although Subgroup 1 is not strictly comparable with the other groups in this respect, there is no indication that the more severe attacks have produced any permanent liver dysfunction.

The course of H.A. synthesis and of serum bilirubin concentration in two patients are shown in the accompanying Chart,

Serum bilirubin values and hippuric acid synthesis in two "typical" cases of infective hepatitis

as illustrating the types of case most often met with. They represent much the same picture as would be obtained from a study of the average figures recorded in the Table.

Mean Values of Serum Bilirubin and Hippuric Acid Synthesis during the Course of the Disease in 81 Cases of Infective Hepatitis

Serum Bilirubin Group	No. of Cases	On Admission		First Week after Admission			Before Discharge			
		H.A.	Serum Bilirubin (mg./100 ml.)	H.A.	Serum Bilirubin (mg./100 ml.)	Days after Admission	H.A.	Serum Bilirubin (mg./100 ml.)	Days after Admission	
A. Rising on admission :										
(1) Maximum above 10 mg./100 ml. 	14	0.527	9.50	0.653	12.45	6.0	1.048	1.61	38.8	
(2) Maximum below 10 mg./100 ml. 	23	0.689	4.64	0.850	5.22	5.5	1.060	1.10	27.2	
Total 	37	0.637	6.50	0.776	7.88	5.7	1.057	1.29	31.6	
B. Falling on admission :										
(3) Admission value above 8 mg./100 ml. 	17	0.678	10.25	0.993	3.86	6.7	1.155	1.05	28.3	
(4) Admission value below 8 mg./100 ml. 	27	0.683	4.61	1.008	1.83	6.3	1.081	0.75	27.0	
Total 	44	0.680	6.79	1.005	2.61	6.4	1.107	0.85	27.4	

H.A. = Hippuric acid synthesis in terms of grammes of sodium benzoate.

Possible Objections to Use of Test

(a) *Renal Damage.*—Although there is no reliable evidence to suggest that kidney damage occurs in any but the most severe cases of infective hepatitis, and Schwei and Quick (1942) have shown that damage to the kidney would have to be considerable before H.A. excretion became a limiting factor in the liver function test, it was thought advisable to test the renal function of a sample of cases in order to obtain first-hand evidence. Blood-urea concentrations and urea-clearance tests were therefore done on 12 unselected cases on admission. In all instances results were well within normal range. The mean blood urea was $26.4 (\pm 1.74)$ mg. per 100 ml., and the mean urea clearance was 159% of average normal, the lowest value being 97%. It does not appear likely, therefore, that renal damage, if it occurs at all, is sufficient to invalidate the use of the H.A. test in infective hepatitis.

(b) *Changing Protein Intake.*—It has been shown by Probst and Londe (1942) that H.A. synthesis can often be increased by ingesting large quantities of glycine. Many patients in this series suffered from anorexia on admission, and in most cases their appetite, and therefore their intake of protein, increased during the first week in hospital. It was calculated that, on an average, the protein intake per day increased from 60 g. (on admission) to 120 g. (on discharge), and a large proportion of this increase probably occurred during the first week. It was therefore considered possible that at least part of the improvement in H.A. synthesis found to occur in most cases of infective hepatitis after admission to hospital might be a reflection of improvement in appetite rather than in liver function. It was accordingly decided to investigate the effect of a severe reduction in protein intake on H.A. synthesis. Twelve convalescent patients were tested shortly before discharge—i.e., at a time when liver function might be considered to be constant or at least not deteriorating—by performing H.A. tests immediately before and after four days of a diet containing no milk, meat, or eggs and very little bread. This entailed a protein reduction from at least 120 g. a day to between 20 and 30 g. a day. The mean H.A. synthesis dropped by 10% from 1.00 to 0.90—a difference of $-0.10 (\pm 0.035)$. However, three patients actually showed a slight increase in H.A. synthesis after the diet. Since the conditions in this experiment are not and never could be really comparable with those pertaining to patients at the height of the disease, it is not possible to draw any hard-and-fast conclusion. The drop in protein intake of nearly 100 g. was possibly rather severe and certainly more than the average increase during the first week in hospital; and there is at present no means of knowing the relative effects of big changes in protein intake on H.A. synthesis of patients with different degrees of liver damage. It is not felt, therefore, that this difference in H.A. synthesis is great enough to invalidate the main conclusions based on our figures, although the results should, on this account, be treated with due reserve.

Discussion

These results show that in most cases there was a progressive improvement in liver function, as judged by the H.A. test, from the time of admission to hospital, although initially in nearly half of them the serum bilirubin concentration and jaundice were actually increasing. The H.A. synthesis was to a large

extent correlated with the severity of the attack and the stage of the disease, but only partly, and indirectly, with the serum bilirubin concentration.

It is possible that the apparent dissociation of bilirubin excretory function and H.A. synthesis might be explained by the fact that bilirubin is being formed in the body at an approximately constant rate, and that the efficiency of the liver is therefore to be judged by the amount of bilirubin excreted each day rather than the concentration in the serum. In other words, so far as bilirubin excretion is concerned, liver function should be gauged by the rate of rise or fall of the serum concentration; it is then clear that even a rising serum bilirubin might be associated with an improving excretion, so long as the rate of rise was diminishing. This is of course an over-simplification of the problem, since it does not take into account the excretion of bilirubin into the urine. The main principle, however, still applies whatever complicating factor is introduced, and may be enough to account for the apparent dissociation of function referred to above—without, of course, being evidence against such dissociation occurring in other respects. It also, perhaps, contributes to an explanation for the oft-repeated but perplexing observation that, in infective hepatitis, the appearance of jaundice frequently coincides with clinical improvement.

The present work has shown that H.A. synthesis was already improving on the patient's admission to hospital, and the final conclusion which seems to be justified is that maximum liver damage occurs in most cases before admission. This is consistent with the finding previously reported (Pollock, 1945) that liver damage is present in the pre-icteric stage some time before the serum bilirubin rises above normal and often several days before the onset of jaundice.

The practical application of this conclusion is that in order to demonstrate satisfactorily the effect of specific treatment in the average case it is preferable that it be started before the liver has begun to improve spontaneously—in effect this means before jaundice appears. Every effort was made to arrange for admission of our patients to hospital as soon as possible after the onset of symptoms; but, even so, only three were admitted in the pre-icteric stage, and it is unlikely that speedier admission will be achieved until there is some outstanding improvement in methods of early diagnosis. The problem of treatment of infective hepatitis depends, more than in most diseases, upon accurate diagnosis in the initial stages.

Summary

The course of liver damage in 81 cases of infective hepatitis has been followed by the use of serial hippuric acid synthesis tests.

The ability to synthesize hippuric acid bore some relation to the severity of the attack and to the stage of the disease, but was only indirectly related to the serum bilirubin level.

In most cases there was progressive improvement in hippuric acid synthesis from the time of admission to hospital, even though it was often associated with a rising serum bilirubin and increasing jaundice.

It is probable that maximum liver damage in this disease most commonly occurs before the patient is admitted to hospital. Specific treatment is therefore not likely to have any striking success unless begun in the pre-icteric stage.

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Sir John Fraser, presiding at a meeting of the technical committee of the Scottish Blood Transfusion Association, reported that in the quarter ended Sept. 30 last the demands of the hospitals for blood and plasma had, for the first time, exceeded the supply of blood obtained from donors. Fortunately, Sir John said, the regional services had sufficient reserves to meet this contingency. There had been a falling off in donor attendances since the end of hostilities—a fact which gave rise to some concern in view of the continuing and increasing demands of the hospitals, particularly the maternity hospitals. During the quarter 6,986 bottles of blood were obtained from donors, as against 11,946 in the preceding quarter; and, but for reserve stocks, this would have been insufficient to meet the 3,934 bottles of blood and 2,937 bottles of plasma supplied to or reserved for the hospitals. An increase in the use of plasma by hospitals was reported at the meeting—a circumstance emphasizing the great need for new blood donors.

MANAGEMENT OF RECENT FRACTURE-DISLOCATIONS OF THE CERVICAL SPINE

BY

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The treatment of fracture-dislocations of the cervical spine by continuous skull traction (Crutchfield, 1933) is well enough established for a detailed discussion of its justification and indications to be unnecessary (but see Key and Conwell, 1937). Descriptions, however, of the practical details of the method vary considerably. The present communication is an attempt to combine various suggestions into a scheme of treatment which, so far as is possible, shall be simple, efficient, and comfortable to the patient.

Application of Traction

Reduction of a fracture-dislocation of the cervical spine involves both traction and extension (Watson-Jones, 1943). These can be achieved simultaneously by an upward pull applied to the skull on a plane anterior to the atlanto-occipital joint. The anterior longitudinal ligament of the spine is thus tautened, and extension of the cervical intervertebral joints follows. This effect has been secured by traction on the zygomatic processes, but that has the disadvantage that extension of the head tends to proceed until the skin of the front of the neck is taut. In this position swallowing is difficult enough for the normal individual; when further impeded by dysphagia of medullary origin or by a large retropharyngeal haematoma it becomes impossible. On the other hand, if the calliper is placed directly above the pinna—i.e., vertically above the atlanto-occipital joint (as recommended by McKenzie, 1935; Barton, 1938; Watson-Jones, 1943)—the force causes traction alone without extension.

Hoer (1936) proposed to place the point of traction slightly more anteriorly, which is mechanically sound. He preferred to use 18- or 20-gauge stainless-steel wire, sinking two pairs of burr-holes in the skull near the midline and running wires through the extradural space between them. This method leaves the sides of the patient's head clear of projecting apparatus and therefore allows him to be turned more easily; the only objection is that the intervening bridge of skull has been known to break during the period of traction. In the series of cases on which this paper is based callipers or steel wire were used as they happened to be available.

Counter-traction

Counter-traction is made by the friction of the patient's body against the mattress on which he lies, and a gravitational component may be added by raising the head of the bed. This latter also reduces the thrust of the abdominal viscera on the patient's diaphragm and his liability to pulmonary congestion and pneumonia. When abdominal distension is severe, as is not uncommon, raising the head of the bed even as much as two feet may not suffice to prevent respiratory distress. McKenzie (1935) illustrates a case of fracture-dislocation, evidently not complicated by spinal cord damage, which was nursed in the sitting position. In this position the upward thrust of the abdominal viscera is minimal and the patient can look round at others in the ward or read without recourse to mirrors or special apparatus. Further, his head is in the most natural position for the act of swallowing. I found that McKenzie's method can equally well be applied to cases with cord lesions.

Management of the Case

In the method here described the point of application of traction is kept slightly anterior to the pinna so as to maintain extension, and the patient is nursed in the sitting position. The apparatus required has been simplified to the degree demanded by Service conditions. The bed is prepared as follows:

A stout post is lashed to the middle of the upper end and a wooden back-rest is laid at an angle of 60° on its upper half. Attach to