THE BLOOD SUGAR IN CASES OF
EPILEPSY.BY
I. DE BURGH DALY, JOHN PRYDE,
M.A. M.D., B.Sc.,
ANDJ. WALKER, M.D., B.Ch., D.P.H.
(From the Physiology Institute, Welsh National School of Medicine,
Cardiff, and Cardiff City Mental Hospital.)

DR. MACKAY's letter¹ leads us to believe that certain experiments which we have performed, and which bear on the question of a possible correlation of hypoglycaemia and epilepsy, will be of interest. Four cases of epilepsy on hospital diet and with no past history of syphilis were selected for investigation. These were under luminal treatment, but this was discontinued before the investigation commenced. Blood was obtained during periods of quiescence except where otherwise stated. The method of investigation involved the withdrawal of 30 to 100 c.cm. of blood from a vein, this large volume being necessary for the combined polarimetric and copper reduction methods which were used for determining the blood sugar.

METHOD.

1. *Estimation of the Blood Sugar by Copper Reduction Method.*—Shaffer and Hartmann's method² was used, since this procedure in our experience is readily applied to a series of parallel determinations and gives reliable and uniform results.

2. *Estimation of the Blood Sugar by the Polarimetric Method.*—The blood filtrates on which this was carried out were obtained essentially by the procedure described by Winter and Smith.³ In certain cases this procedure was followed exactly as described by these authors; in other cases three extractions with 85 per cent. alcohol were carried out instead of two, and the final polarimetric solution was made up to a volume less than that of the corresponding amount of the original blood used. By this means a solution was obtained which contained the sugar in a higher con-

CASE I.—H. H. L. Blood taken on June 12th and 15th, 1923. Patient had one fit on June 13th. Luminal discontinued on the morning of June 12th. Not confined to bed.

Date.	A. Percentage of Sugar (as dextrose) in Blood. Shaffer-Hartmann.	B. Percentage of Sugar (as dextrose) by Polarimetric Method.	C. Copper Reduction Value of B (as dextrose). Shaffer-Hartmann.
12.6.23	0.085	0.036	—
13.6.23	—	0.077	—
14.6.23	—	—	0.075
15.6.23	0.080	Undetermined since polarimetric solution was initially laevo-rotatory. A Shaffer-Hartmann determination gave 0.014 per cent. reducing substance as dextrose.	—

CASE II.—E. L. Blood taken on June 12th, 15th, and 26th, 1923 (ten minutes after a severe fit). Two fits on June 13th. Succession of six fits on June 25th. Carbuncle on neck, confined to bed.

12.6.23	0.065	—	—
13.6.23	—	0.029	—
14.6.23	—	0.025	0.036
15.6.23	0.080	Undetermined since polarimetric solution was initially laevo-rotatory.	—
16.6.23	—	—	—
18.6.23	—	0.029	—
13.6.23	—	0.036	—
21.6.23	—	0.103	0.076
26.6.23	0.10	0.031	—
27.6.23	—	0.044	—
28.6.23	—	0.010	—
30.6.23	—	0.019	—
3.7.23	—	0.02	0.065

CASE III.—A. R. Blood taken on June 25th, 1923 (twenty minutes after a fit).

Date.	A. Percentage of Sugar (as dextrose) in Blood. Shaffer-Hartmann.	B. Percentage of Sugar (as dextrose) by Polarimetric Method.	C. Copper Reduction Value of B (as dextrose). Shaffer-Hartmann.
25.6.23	0.085	Undetermined since polarimetric solution was initially laevo-rotatory.	—
27.6.23	—	Ditto	—
28.6.23	—	Ditto	—
29.6.23	—	Ditto	—
3.7.23	—	—	0.077

CASE IV.—W. J. W. Blood taken on June 26th, 1923 (fifteen minutes after a fit).

23.6.23	0.120	—	0.084
---------	-------	---	-------

centration than that of the original blood, thus rendering more accurate polarimetry possible. With the view of controlling this second method, the copper reducing value of the polarimetric solution was also determined by the Shaffer-Hartmann method as used for the estimation of the sugar in the original whole blood. This was carried out after the polarimetric observations, which extended over a few days, were complete.

The solutions used in the Shaffer-Hartmann determinations were standardized from time to time during the course of the investigations. This we found to be necessary. Two blood sugar determinations on non-epileptic patients (one a case of mania, the other of melancholia) were carried out as controls. These gave respectively 0.107 and 0.105 per cent. blood sugar as dextrose.

Even in this short series of experiments it will be seen that the blood sugar values, as determined directly on the blood by the Shaffer-Hartmann method, while in general within the normal range, nevertheless tend towards the lower limits. In Case ii (June 12th, 1923) the blood sugar percentage was definitely subnormal, but without further investigation we do not care to stress this point.

Turning to the results given by the polarimetric method of determining the blood sugar, there are several points which we consider worthy of note. It will be seen that in three experiments the "blood sugar" filtrate showed a laevo-rotation, which slowly changed to a dextro-rotation in one, and remained more or less laevo-rotatory in the other two cases. The copper reducing values of the polarimetric solution (C) are in general lower than that of the whole blood (A), and in addition the polarimetric method of determining the dextrose present in the solution gave results not in accord with those given by the copper reduction method (B and C). (Compare also Eadie.⁴)

These results, taken into consideration with similar observations by ourselves (unpublished) and other workers⁴ on animals, do not lead us to believe that the changes recorded above are peculiar to cases of epilepsy. Rather, in our opinion, does the explanation of these phenomena depend on the unsuitability of the method itself for dealing with the question of the quantitative and qualitative nature of the blood sugar; as a result of our experiences we believe that the conclusions drawn by Winter and Smith³ from their observations are unjustifiable. We would emphasize the fact that all calculations based on the assumption that dextrose is the only copper reducing and optically active substance present in the final polarimetric solution are fraught with danger. Further, no conclusive evidence has yet been brought forward to show that dextrose is the only carbohydrate present in whole normal blood, apart from the question of the presence of α , β , or γ forms or mixtures of these. Certain of our results may in part be ascribed to loss of reducing substance by imperfect extraction, and the liability of such an occurrence appears to be a fault of the method. In conclusion, attention may be drawn to the fact that it has recently been shown by Stasiak⁵ that the sugar content, as determined by copper reduction methods, of blood serum, free from enzymes and kept *in vitro*, shows irregular variations, and that similar variations occur in pure solutions of dextrose containing globulin and cholesterol. There is no evidence to show that in the method of Winter and Smith

the elimination of these and other possible interfering substances is complete, and it is suggested that inconsistent and variable results obtained in determining the sugar present in blood filtrates are due to such phenomena as those recorded by Stasiak. In confirmation of these observations we have found (unpublished research) that the copper reducing value of the polarimetric solution obtained from dog's blood by the method of Winter and Smith exhibits irregular variations from day to day.

We have pleasure in expressing our thanks to Dr. Edwin Goodall for his kindness in allowing us access to clinical material at the Cardiff City Mental Hospital. One of us (J. P.) is indebted to the Medical Research Council for a grant.

REFERENCES.

¹ Mackay: BRITISH MEDICAL JOURNAL, December 15th, 1923. ² Shaffer and Hartmann: *Journ. Biol. Chem.*, 1921, xlv, 2, 365. ³ Winter and Smith: *Journ. Physiol.*, 1922, lvii, 100. ⁴ Eadie: BRITISH MEDICAL JOURNAL, July 14th, 1923. ⁵ Stasiak: *Biochemische Zeitschrift*, 1923, cxi, 420.

A COMPARISON OF VARIOUS FORMS OF COLLOIDAL FERRIC HYDROXIDE.

BY

SIR WILLIAM J. POPE, AND R. T. M. HAINES,
F.R.S., M.A.

(From the Chemical Laboratory, University of Cambridge.)

FERRIC hydroxide, $\text{Fe}(\text{OH})_3$, is readily obtained in colloidal solution by a variety of methods, the products of which differ in a number of important respects. It has long been recognized that several distinct modifications of colloidal ferric hydroxide exist and it may be of interest to record a number of points in connexion with their physical and chemical behaviour and their reactions towards physiological fluids.

The several so-called soluble forms of ferric hydroxide contain the latter substance as a suspensoid colloid—namely, in the form of submicroscopic particles suspended in a liquid medium. For purposes of convenience the colloidal ferric hydroxides may be ranged in two categories according as the suspensoid particles carry a positive or a negative electric charge; this classification is useful because many of the properties of colloidal solutions are determined by the sign of the electric charge of the suspensoid particles. The present note deals with the comparison of the four typical colloidal ferric hydroxide solutions next described.

A. The best known of the positively charged ferric hydroxide suspensoids was prepared by Graham in 1861 by dissolving ferric hydroxide in aqueous ferric chloride solution and subjecting the resulting solution to dialysis, ordinary parchment paper being used as the diaphragm; the solution thus prepared constitutes the liquor ferri dialysati of the old *Pharmacopoeia*. Under these conditions hydrochloric acid passes through the membrane into the surrounding water and the dialyser retains a deep brown-coloured colloidal solution of ferric hydroxide; the colloid always contains a small proportion of the acid radicle originally present in the ferric salt, but this, by prolonged dialysis, can be reduced to a minimum. In this way Duclaux reduced the percentage of chlorine until it corresponded with the presence in the colloidal solution of aggregates of the composition $450\text{Fe}(\text{OH})_3 \cdot \text{FeCl}_3$. The preparation used in the present investigation was the ordinary pharmaceutical material of commerce.

B. Another, and quite distinct, colloidal ferric hydroxide solution is prepared by blowing steam through a boiling aqueous solution of ferric formate until the escaping vapour gives no acid reaction. This solution is much deeper in colour than one of the same iron content of the dialysed

iron A, and is notably less stable towards the action of precipitating agents.

C. If a solution of ferric formate is heated to 100° for some hours and then submitted to prolonged dialysis a very pale-coloured colloidal ferric hydroxide solution is obtained which is still more readily coagulated. This colloid is remarkable in that the ferric hydroxide precipitated from it is of a light yellow colour and is apparently in a higher state of hydration than the red-coloured ferric hydroxide which separates from solutions A and B.

In the three above preparations the suspended particles of ferric hydroxide carry a positive electric charge, as may be shown by passing an electric current through the solution, when the ferric hydroxide particles travel towards the negative electrode or cathode.

D. Colloidal ferric hydroxide solutions in which the particles carry a negative electric charge were also prepared by Graham in 1861 by the dialysis of ferric chloride solutions containing sugar; such solutions have been since found to be more conveniently prepared by the dialysis of solutions of ferric chloride with glycerol or sugars, to which caustic soda has been added. In these negatively charged forms of colloidal ferric hydroxide solution the sugar or glycerol acts as a protective and prevents the immediate coagulation of the ferric hydroxide; the protectives named do not, however, render the colloidal solutions permanently stable, and other protectives such as gelatin must be added to ensure stability. The preparation now issued as collosol ferrum is a colloidal ferric hydroxide solution protected in the manner just indicated and is used in the comparative experiments described below.

The four solutions used throughout the experiments now described were of the same concentration in iron, containing 0.5 gram of iron per litre; the concentration in iron may thus be stated as 0.05 per cent.

The results recorded in Table I show that the three forms of positive colloidal ferric hydroxide differ considerably in stability towards electrolytes and ordinary reagents; it is evident that the liquor

ferri dialysati is more stable than the other two, and is, in fact, the only one of the three which is not coagulated by freezing at -40°C . and subsequent thawing. The collosol ferrum is, however, considerably more stable than the liquor ferri dialysati and gives no indication of the presence of a soluble ferric salt by such delicate tests as those with ferrocyanide, thiocyanate, resorcinol, or

salicylic acid; it is the only one which is not coagulated by addition of colloidal arsenic sulphide.

The ultramicroscopic examination of these colloidal solutions at various degrees of dilution shows them to be very different in colloidal character. The steamed ferric formate solution, B, and the collosol ferrum, D, show numerous particles (submicrons) upon a dark background; many more submicrons are present in solution D than in B and the background is slightly the lighter in the case of D, indicating the presence of a higher concentration of amicroscopic particles. The liquor ferri dialysati, A, shows but few submicrons upon a light background, this latter indicating the presence of numerous amicros. The dialysed ferric formate solution, C, contains very few particles or submicrons, but shows intense general illumination of the background, due to the presence of large numbers of amicros.

A rough count shows that the collosol ferrum solution contains about 2×10^{10} submicrons per cubic centimetre. The relative numbers of particles or submicrons, the indicated relative concentrations of the amicros, and the depth of colour of the four solutions are also given in Table I. A comparison of these particulars shows that these colloidal ferric hydroxide solutions follow Ostwald's rule that the colour deepens as the size of the particles increases.