dence of rickets, since 12.8 per cent of 349 infants examined in October showed very slight rickets, and 4.5, mild rickets. No infants however showed moderate or marked rickets.

4. Infants under 4 months of age in October are more apt to develop rickets in the succeeding five winter months than are infants from 4 to 8 months of age in October.

5. One, 2 or 3 teaspoonfuls of cod liver oil were prescribed for 137 Toronto infants, largely of British or northern European descent. Three infants developed moderate or marked rickets during a period of five winter months. Difficulties encountered in the administration of cod liver oil under home conditions may have accounted for the development of rickets in these infants.

6. One teaspoonful of cod liver oil was at least as efficacious as 3 teaspoonfuls in the prevention of rickets, as evidenced by x-rays.

7. One hundred, 200, 400 and 800 Steenbock vitamin D units, in the form of viosterol (1.5 to 12 drops of 250D viosterol), were prescribed for 186 Toronto infants, largely of British or northern European descent. Under these conditions no infants developed moderate or marked rickets during a period of five winter months.

8. One hundred, 200, 400 and 800 units, in the form of viosterol (from 1.5 to 12 drops of 250D viosterol), were efficacious to the same degree in the prevention of rickets, as evidenced by x-rays.

9. From 20 to 40 ounces of irradiated vitamin D milk, containing 35 Steenbock vitamin D units per 20 ounces, were prescribed to 141 Toronto infants ranging in age at initial examination from 3 weeks to 8 months, and largely of British or northern European descent. The milk was administered over a period of five winter months. Under these conditions no infants developed moderate or marked rickets.

10. In irradiated vitamin D milk we have a valuable addition to our present anti-rachitic armamentarium.

We desire to express our appreciation of the co-operation and assistance given us by Dr. Gordon Jackson, Medical Officer of Health, City of Toronto. We also wish to thank the various physicians in the well baby clinics who have cooperated so splendidly in this work.

REFERENCES

1. Report of Council on Pharmacy and Chemistry, Am. Med. Association, *J. Am. M. Ass.*, 1932, **98**: 316.
2. Report of Council on Pharmacy and Chemistry, Am. Med. Association, *J. Am. M. Ass.*, 1929, **93**: 693.
3. Report of Council on Pharmacy and Chemistry, Am. Med. Association, *J. Am. M. Ass.*, 1930, **95**: 1021.
4. HESS, A. F. AND LEWIS, J. M.: Appraisal of antirachitics in terms of rat and clinical units, *J. Am. M. Ass.*, 1933, **101**: 181.

# A CONVENIENT METHOD FOR DETERMINING SERUM AND BILE PHOSPHATASE ACTIVITY*

BY EARL J. KING AND A. RILEY ARMSTRONG,

*Department of Medical Research, Banting Institute, University of Toronto, Toronto*

CLINICAL laboratories are at the present time frequently being called upon to make estimations of the phosphatase activity of blood serum. This demand has followed upon the realization of the diagnostic aid which such a determination may lend in cases of both bone disease (Kay;[6, 7] Roberts[9]) and jaundice (Roberts;[10] Armstrong, King and Harris[1]).

Robison[11] described the presence of phosphatase in bone and ossifying cartilage and in blood (Robison, Hansman and Martland[12]) and outlined a method for determining the relative enzyme content of a solution. While considerable progress has been made since then in diminishing the time required for such a determination, present methods are as yet too time-consuming and laborious for clinical work.

Since the enzyme has not been obtained in a pure state, the amount present in a given solution is expressed in terms of its ability to effect hydrolysis on a given substrate. Such ability is known as phosphatase activity and is expressed in arbitrary units.

The fundamental basis of determining phosphatase activity has been the same in all methods to date. It has consisted in measuring the phosphorus set free as inorganic phosphate when the enzyme is allowed to act on a substrate (disodium B glycerophosphate) under standard conditions. An example of a representative method adapted to clinical needs is that of Jenner and Kay.[5] Using the procedure of these

* Received for publication June 6, 1934.

authors, 5 c.c. of blood are required from the patient to supply enough plasma for duplicate analyses. The time required for a complete determination is about 5 hours, and the accuracy is well beyond that required clinically. Similar procedures, in which the hydrolysis has been conducted over shorter intervals, have been described by Roberts[9] and by Bodansky.[3] Unfortunately, the units of phosphatase activity adopted by the different authors have not been the same. Thus the results reported, which comprise a large and valuable series, cannot readily be compared.

The objects in view when the present study was commenced were three: firstly, to elaborate a more rapid and, if possible, more accurate method than those previously described; secondly, to limit the total amount of serum required for duplicate test and control hydrolysis to 2 c.c.; and, thirdly, to express the relative amount of phosphatase found in units numerically equal to those of Jenner and Kay.* Fortunately, by employing a different substrate it has been found possible to fulfil all three objects set forth above. The new unit represents almost exactly the same amount of enzyme as the former one. The definition of that unit is, however, entirely different.

King[8] observed that the rate at which di-sodium phenyl phosphate is hydrolyzed by phosphatase was approximately double the rate for di-sodium B glycerophosphate. Theoretically, therefore, by substituting the former substrate for the latter in the Jenner and Kay method, the time required for hydrolysis could be reduced to about one and a half hours. The time of hydrolysis was further reduced by taking advantage of the fact that the estimation of the phenol liberated gives the same measure of the extent of hydrolysis as does the estimation of the liberated phosphate. In a given weight of phenyl phosphate there is approximately three times as much phenol, weight for weight, as there is phosphorus, so that after a hydrolysis lasting 30 minutes there would be as much phenol liberated by weight as there would be phosphorus at the end of one and a half hours. Hence, if small amounts of phenol could be measured with the same degree of accuracy as similar amounts of phosphorus, then the time for hydrolysis in the above substitution could be limited to 30 minutes. Fortunately, an accurate procedure for estimating phenol was available.

The phenol method of Benedict and Theis[2] was tried out, since it offers the advantage of being fairly specific and the reagent is easily prepared. However, the colours were difficult to read in the colorimeter and turbidities were troublesome. Folin and Ciocalteu[4] described a reagent (a phosphotungstic phosphomolybdic acid) for determining tryptophane and tyrosine in blood, which forms a blue colour with phenol when made alkaline. This reagent, although it takes some time to prepare, was found to be very adaptable to our purpose, and it possesses the advantage of being a good protein precipitant. The colours can be read with great ease in the colorimeter and turbidities do not develop. It was finally adopted after some necessary modifications.

The cumbersome use of the several substances required for glycine-sodium hydroxide-sodium chloride buffer was avoided by the use of the veronal buffer of Michaelis. Jenner and Kay had avoided this buffer since they had trouble with crystallization when the solution was acidified. Bodansky[3] claimed never to have had this experience. However, his buffer solution is so weak that it must be handled with great care and kept under a layer of paraffin, lest carbon dioxide from the air lower the pH. Employing a much stronger veronal solution, we have found that the pH of the buffer solution thus prepared is remarkably constant. Some of the samples of veronal tested have crystallized out when made faintly acid; others have not. The cause for this we do not know, but it is easy to secure a suitable sample and then make further purchases of material made in the same batch.

For the sake of convenience both buffer and substrate are made up in one solution and preserved with a few drops of chloroform in the ice-chest. If there is any suspicion that hydrolysis may have occurred in the solution after standing, this point may be briefly settled by adding to a few c.c. of it a little of the phenol reagent and making it alkaline. Only a trace of blue colour should develop.

Serum and oxalated plasma were employed interchangeably, since we found that there were no appreciable differences in the results ob-

* One Jenner and Kay unit represents that amount of enzyme which will liberate one mg. of phosphorus as free phosphate from an excess of di-sodium B glycerophosphate at pH 8.8 in 3 hours, when kept at a temperature of 37.5° C.

tained. Bodansky (1933) also employed serum in preference to plasma.

Under the conditions of the test employed, 1 unit of phosphatase is that amount of enzyme which, when allowed to act upon excess di-sodium phenylphosphate at pH 9.0 for 30 minutes at 37.5° C., will liberate 1 mg. phenol.

SOLUTIONS REQUIRED

1. *Buffer Substrate.*—Molar/200 phenyl phosphate in molar/20 veronal.

10.3 g. sodium veronal.
1.09 g. di-sodium phenylphosphate.*
Water to 1 litre.

Preserve in a well-stoppered bottle with a few drops of chloroform, and keep in the ice-chest when not in use. (This solution keeps at least 1 month).

2. *Phenol Reagent of Folin and Ciocalteu.*†—As described in Peters and Van Slyke "Quantitative Clinical Chemistry", **2:** 655, and in Hawk and Bergeim, 10th Edition, p. 866.

Dilute this reagent 1 in 3.

3. *20% solution of sodium carbonate ($Na_2CO_3$).*

4. *Stock Standard Phenol (approximately 100 mg. per 100 c.c. solution of phenol).*—Dissolve 1 g. crystalline phenol in 0.1 N HCl and make up to 1 litre with 0.1 N HCl. Titrate with iodine as described in Peters and Van Slyke, "Quantitative Clinical Chemistry", **2:** 655, and in Hawk and Bergeim, 10th Edition, p. 866, and note the exact strength. (This solution keeps indefinitely).

5. *Diluted Stock Standard Phenol (exactly 10 mg. per 100 c.c. solution of phenol.*—Made by a suitable dilution of (4). (This keeps at least 3 months in the ice-box).

6. *Standard phenol solution and reagent (1 mg. phenol per 100 c.c.).*—

5 c.c. diluted stock standard (10 mg. per 100 c.c.).
15 c.c. diluted phenol reagent.
Water to 50 c.c.
(Make up daily).

PROCEDURE

*Blood.*—Five c.c. of blood are drawn from an arm vein in the usual manner and without the addition of anti-coagulant. After the clot has formed it is loosened from the sides of the tube and then centrifuged. The serum is poured off into a centrifuge tube, and after being again well centrifuged to get rid of any suspended cells is ready for use. If the determination cannot be done on the day the blood is taken, the blood should be preserved in the ice-box over night. The results obtained on sera before and after 24 hours' preservation in the ice-box agree very closely.

*Test.*—In 2 test tubes place 10 c.c. of buffer substrate. Allow the tubes to remain in a water bath at 37.5° C. for 5 minutes or more. Without removing the tubes from the bath, add exactly 0.5 c.c. serum to each, stopper, mix, and allow to remain in the bath exactly 30 minutes. At the end of this time add at once 4.5 c.c. of dilute phenol reagent, mix, and filter.

*Control.*—In 2 test tubes place 10 c.c. buffer substrate. Add to each 0.5 c.c. serum and at once 4.5 c.c. diluted phenol reagent and filter. Pipette 10 c.c. filtrate from the test and control solutions into clean test tubes. Add 2.5 c.c. of 20 per cent sodium carbonate, mix, and replace tubes in the water bath for five minutes to bring up the colour.

*Comparison.* — Compare in the colorimeter with a standard made up at the same time by taking 10 c.c. of standard phenol solution and reagent (No. 6 above), and 2.5 c.c. of 20 per cent sodium carbonate. Place the unknown solution on the left side of the colorimeter, and set at 30 mm. Place the standard on the right side and match the colours.

*Calculation.*—The phosphatase activity of a serum is expressed as units per 100 c.c. of serum and is numerically equal to the mg. of phenol which would be set free from the phenyl phosphate under the standard conditions by 100 c.c. of serum. Thus:

$$\text{Units of phosphatase per 100 c.c.} = \frac{\text{mg. phenol per 100 c.c. serum in test}}{} - \frac{\text{mg. phenol per 100 c.c. serum in control}}{}$$

The number of mg. phenol in 100 c.c. of serum in the test and in the control is found by the equation:

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times \text{Strength of Standard} \times \frac{15}{10} \times \frac{100}{0.5}$$

With the unknown solution set at 30 mm. and the strength of standard = 0.1 mg. this equation can be written:

$$\frac{\text{Reading of Standard}}{30} \times 0.1 \times \frac{15}{10} \times \frac{100}{0.5}$$

All figures in the above equation cancel out, making it equal to "Reading of Standard".

More simply, then:

$$\text{Units of phosphatase per 100 c.c.} = \frac{\text{Reading of Standard (against the Test)}}{} - \frac{\text{Reading of Standard (against the Control)}}{}$$

*Procedure when the enzyme content of the serum is very high.*—So long as the enzyme content of the serum is less than 30 units the colorimetric reading is carried out as described above. If more than 30 units are present, but less than

* Di-sodium-monophenylphosphate may be purchased from British Drug Houses, Limited.

† Phenol Reagent of Folin and Ciocalteu may be purchased from the J. F. Hartz Company, Toronto.

60 units, the reading of the unknown for the test solution should be set at 15 mm. In such a case "Reading of Standard" (against test) must then be multiplied by 2 to obtain mg. phenol per 100 c.c. of serum. When the enzyme content of serum is very high, the products of hydrolysis will become correspondingly greater in amount. These, especially the phosphate, have a retarding influence on the velocity of the hydrolysis when a certain concentration is reached. By experimentation we have found that if less than 60 units of phosphatase are present dilution of the serum yields the same final result as is obtained in the undiluted serum. However, if the serum contains above 100 units very great differences are found between diluted and undiluted serum. Two such experiments gave the following results.

| | *Case 1* | *Case 2* |
|---|---|---|
| Undiluted ..... | 123 units | 118 units |
| Diluted with inactivated serum | | |
| 1 to 3 ..... | 202 " | 170 " |
| 1 to 9 ..... | 199 " | 165 " |
| " " saline 1 to 3 ..... | 206 " | 183 " |

For routine work it is our custom to dilute high phosphatase sera with normal saline, so that the number of units per 100 c.c. of diluted serum will not be in excess of 60.

The pH of the buffer substrate colorimetrically by B.D.H. capillator = 9.6 at room temperature. After addition of serum the pH = 9.3 – 9.5, (checked by potentiometric measurement). The actual pH at 37.5° C. would thus be pH 8.9 – 9.1.

## THE ACCURACY OF THE METHOD

The 30 minute incubation period as used in this method is of advantage in that it gives results which more truly represent the actual enzyme content of the blood than is the case with methods requiring a longer period of incubation. Theoretically, a proper measurement of the amount of the enzyme should be based on the initial velocity of hydrolysis. While the attainment of this ideal condition is not practicable in a clinical method, the shortening of the period of hydrolysis, without any sacrifice in the accuracy of the analytical procedures, should make for the attainment of more exact and hence more significant values.

When large numbers of sera are being examined routinely we have found duplicate analyses to agree on the average to within 5 per cent for those values lying within the normal range. By exercising more care this error can be cut down very considerably. For the higher values the percentage error is correspondingly less. (For the results of a series of consecutive cases see Table I).

TABLE I.

CONSECUTIVE SERIES OF CASES EXAMINED OVER A PERIOD OF TWO WEEKS SHOWING CLOSE AGREEMENT OF DUPLICATES. (ARRANGED IN ORDER OF ACTIVITY.)

| | *Control (zero time)* | | | *Test (30 minutes incubation)* | | | *Units* | | |
|---|---|---|---|---|---|---|---|---|---|
| *No.* | *(1)* | *(2)* | *Difference* | *(1)* | *(2)* | *Difference* | *(1)* | *(2)* | *Difference* |
| 1 | 7.5 | 7.5 | 0 | 10.9 | 10.7 | 0.2 | 3.4 | 3.2 | 0.2 |
| 2 | 7.1 | 7.1 | 0 | 11.4 | 11.4 | 0 | 4.3 | 4.3 | 0 |
| 3 | 7.1 | 6.9 | 0.2 | 12.5 | 11.6 | 0.9 | 5.4 | 4.7 | 0.7 |
| 4 | 7.5 | 7.5 | 0 | 12.9 | 12.5 | 0.4 | 5.4 | 5.0 | 0.4 |
| 5 | 6.8 | 7.1 | 0.3 | 12.9 | 12.5 | 0.4 | 6.1 | 5.4 | 0.7 |
| 6 | 6.4 | 6.2 | 0.2 | 13.0 | 12.9 | 0.1 | 6.6 | 6.7 | 0.1 |
| 7 | 7.2 | 7.2 | 0 | 13.2 | 13.8 | 0.6 | 6.0 | 6.6 | 0.4 |
| 8 | 6.4 | 6.2 | 0.2 | 14.1 | 13.9 | 0.2 | 7.7 | 7.7 | 0 |
| 9 | 6.9 | 7.1 | 0.2 | 15.5 | 15.4 | 0.1 | 8.6 | 8.3 | 0.3 |
| 10 | 7.3 | 7.6 | 0.3 | 15.6 | 15.9 | 0.3 | 8.3 | 8.3 | 0 |
| 11 | 7.4 | 7.4 | 0 | 16.6 | 16.7 | 0.1 | 9.2 | 9.3 | 0.1 |
| 12 | 7.4 | 7.4 | 0 | 16.7 | 19.7 | 3.0 | 9.3 | 12.3 | 3.0 |
| 13 | 6.6 | 6.5 | 0.1 | 18.1 | 18.6 | 0.5 | 11.5 | 12.1 | 0.6 |
| 14 | 7.5 | 7.5 | 0 | 19.0 | 18.6 | 0.4 | 11.5 | 11.1 | 0.4 |
| 15 | 7.9 | 7.5 | 0.4 | 20.3 | 21.3 | 1.0 | 12.4 | 13.8 | 1.4 |
| 16 | 7.7 | 7.7 | 0 | 20.6 | 19.5 | 1.1 | 12.9 | 11.8 | 1.1 |
| 17 | 7.5 | 7.7 | 0.2 | 22.9 | 22.7 | 0.2 | 15.4 | 15.0 | 0.4 |
| 18 | 7.6 | 7.7 | 0.1 | 23.3 | 23.4 | 0.1 | 15.7 | 15.7 | 0 |
| 19 | 7.2 | 7.2 | 0 | 24.5 | 25.0 | 0.5 | 17.3 | 17.8 | 0.5 |
| 20 | 9.4 | 9.2 | 0.2 | 25.0 | 25.0 | 0 | 15.6 | 15.8 | 0.2 |
| 21 | 7.3 | 7.4 | 0.1 | 25.3 | 24.9 | 0.4 | 18.0 | 17.5 | 0.5 |
| 22 | 5.8 | 5.9 | 0.1 | 25.5 | 24.7 | 0.8 | 19.7 | 18.8 | 0.9 |
| 23 | 9.5 | 9.7 | 0.2 | 35.1 | 35.3 | 0.2 | 25.6 | 25.6 | 0 |
| 24 | 8.9 | 8.9 | 0 | 35.4 | 35.9 | 0.5 | 26.5 | 27.0 | 0.5 |
| 25 | 8.3 | 8.1 | 0.2 | 38.3 | 38.4 | 0.1 | 30.0 | 30.3 | 0.3 |
| 26 | 7.5 | 7.7 | 0.2 | 47.2 | 46.4 | 0.8 | 39.7 | 38.7 | 1.0 |
| 27 | 6.3 | 6.3 | 0 | 73.6 | 73.6 | 0 | 67.3 | 67.3 | 0 |
| 28 | 8.9 | 9.1 | 0.2 | 89.4 | 90.3 | 0.9 | 80.5 | 81.3 | 0.8 |
| 29 | 5.9 | 5.7 | 0.2 | 92.1 | 91.5 | 0.6 | 86.2 | 85.8 | 0.4 |
| 30 | 6.0 | 6.0 | 0 | 96.6 | 95.1 | 1.5 | 90.6 | 89.1 | 1.7 |
| 31 | 8.9 | 8.9 | 0 | 119.7 | 119.7 | 0 | 110.8 | 110.8 | 0 |

TABLE II.

COMPARISON OF UNITS OF NEW METHOD WITH THOSE OF JENNER AND KAY

| *No.* | *Source of Serum* | *Jenner and Kay* | *New Method* | *Difference* |
|---|---|---|---|---|
| 1 | Dog (2) | 3.1 | 6.0 | +2.9 |
| 2 | Rabbit (p) | 5.9 | 6.7 | +0.8 |
| 3 | Dog (A) | 6.1 | 7.4 | +1.3 |
| 4 | Rabbit (2) | 6.1 | 6.9 | +0.8 |
| 5 | " (3) | 6.4 | 9.3 | +2.9 |
| 6 | Human (P) | 6.5 | 8.0 | +1.5 |
| 7 | " (A) | 6.8 | 6.8 | +0.0 |
| 8 | Dog (p) | 6.8 | 7.4 | +0.6 |
| 9 | Rabbit (1) | 6.9 | 8.5 | +1.6 |
| 10 | Human (M) | 8.6 | 9.1 | +0.5 |
| 11 | " (O) | 9.2 | 9.4 | +0.2 |
| 12 | Sheep | 9.2 | 11.3 | +2.2 |
| 13 | Dog (1) | 9.3 | 9.8 | +0.5 |
| 14 | Human (1) | 9.7 | 10.0 | +0.3 |
| 15 | " (R) | 9.7 | 9.5 | −0.2 |
| 16 | " (J) | 12.8 | 12.5 | −0.3 |
| 17 | Dog (3) | 14.4 | 13.3 | −1.1 |
| 18 | Human (B) | 17.7 | 19.7 | +2.0 |
| 19 | " (A) | 29.0 | 26.6 | −2.4 |

A number of sera were examined simultaneously by our own method and by that of Jenner and Kay (Table II). Between the values of 3 to 30 the two methods agree very closely, and most of the differences are scarcely beyond the combined experimental error of both methods. If the serum is diluted before analysis there is considerable divergence. In such cases the new method gives distinctly higher values. The cause for this has not been fully investigated, but addition of Mg.++ ions to both our substrate and to Jenner and Kay's leads to a marked lessening of this difference.

### RANGE OF THE NORMAL IN THE NEW METHOD

The range of units of serum phosphatase found by the new method in 24 apparently healthy laboratory workers is presented in Table III. The ages range from 19 to 42 years. All values lie between 3.7 and 13.1 units; the great majority lie between 5 and 10 units. There is no obvious difference between males and females. Estimations repeated on individuals on different occasions (indicated by letter A) showed little change beyond that to be expected due to experimental error in the low range. On the basis of these findings it would seem advisable to look with suspicion upon any values below 3.0 units or above 13.0 units. The estimations made on dog sera indicated a somewhat wider range of values than was found in the human cases. Two of the twenty-one dogs (No. 20 and No. 21) presented remarkably high values — too high, perhaps, to be regarded as normal. However, we were unable to detect any abnormality which might account for this, and thus have included them in the Table. The sheep and the rabbits examined had values well within the range given for human beings.

TABLE III.

SERUM PHOSPHATASE IN HEALTHY LABORATORY WORKERS AND APPARENTLY HEALTHY LABORATORY ANIMALS

| No. | Initial | Age | Sex | Units of Phosphatase | No. | Initial | Age | Sex | Units of Phosphatase |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M.D. | 24 | F | 3.7 | 14 | H.P. | 29 | M | 8.0 |
| 2 | H.T. | 24 | F | 4.8 | 15 | G.M. | 19 | F | 8.0 |
| 3 | H.E. | 37 | M | 5.4 | 16 | F.N. | 32 | M | 8.2 |
| 1A | M.D. | 24 | F | 5.5 | 17 | E.K. | 32 | M | 8.2 |
| 4 | G.C. | 40 | M | 5.8 | 18 | G.O. | 30 | M | 8.3 |
| 5 | L.P. | 26 | M | 5.9 | 17A | E.K. | 32 | M | 8.9 |
| 6 | K.W. | 24 | M | 6.0 | 19 | M.M. | 28 | M | 9.1 |
| 7 | W.F. | 33 | M | 6.0 | 20 | F.B. | 42 | M | 9.2 |
| 8 | M.W. | 24 | F | 6.1 | 18A | G.O. | 30 | M | 9.4 |
| 6A | K.W. | 24 | M | 6.5 | 21 | J.R. | 31 | M | 9.5 |
| 9 | B.B. | 27 | M | 6.6 | 22 | D.I. | 34 | M | 10.0 |
| 10 | R.A. | 29 | M | 6.8 | 23 | J.F. | 34 | M | 10.0 |
| 11 | E.H. | 26 | M | 7.0 | 5A | L.P. | 26 | M | 11.1 |
| 12 | A.S. | 28 | F | 7.3 | 24 | E.B. | 24 | F | 11.7 |
| 13 | A.M. | 27 | M | 7.7 | 23A | J.F. | 34 | M | 13.1 |
| 1 | Dog | | | 1.9 | 11 | Dog | | | 7.4 |
| 2 | " | | | 2.3 | 12 | " | | | 9.0 |
| 3 | " | | | 2.4 | 13 | " | | | 9.8 |
| 4 | " | | | 3.4 | 14 | " | | | 10.1 |
| 5 | " | | | 4.5 | 15 | " | | | 10.4 |
| 6 | " | | | 4.6 | 16 | " | | | 10.9 |
| 7 | " | | | 4.7 | 17 | " | | | 11.5 |
| 8 | " | | | 5.6 | 18 | " | | | 13.3 |
| 9 | " | | | 6.0 | 19 | " | | | 15.7 |
| 10 | " | | | 6.6 | 20 | " | | | 27.4 |
| | | | | | 21 | " | | | 32.1 |
| | Sheep | | | 11.3 | | | | | |
| 1 | Rabbit | | | 6.9 | | | | | |
| 2 | " | | | 8.5 | | | | | |
| 3 | " | | | 9.3 | | | | | |

TABLE IV.

SERUM PHOSPHATASE IN VARIOUS CLINICAL CONDITIONS

| Case No. | Diagnosis | Serum Phosphatase |
|---|---|---|
| 1 | Latent parathyroid tetany | 6.9 |
| 2 | Active parathyroid tetany | 7.0 |
| 3 | Fractured femur | 12.1 |
| 4 | Pott's fracture | 10.7 |
| 5 | Fragilitas ossium | 23.3 |
| 6 | Multiple myelomata | 14.9 |
| 7 | Hyperparathyroidism— Rarefaction of bones | 67.5 |
| | (1 month later) | 121.0 |
| 8 | Fibrocystic disease | 110.0 |
| 9 | Cholecystitis and cholelithiasis without obstruction | 28.8 |
| 10 | Infectious jaundice | 36.7 |
| 11 | Obstructive jaundice | 65.0 |
| 12 | Obstructive jaundice | 162.0 |
| 13 | Acute hæmorrhagic pancreatitis | 6.5 |
| 14 | Baby, aged 28 months; convalescent from pneumonia | 19.0 |
| 15 | Baby, aged 9 months; scurvy | 23.0 |
| 16 | Baby, aged 12 months; craniotabes | 90.0 |
| 17 | Baby, aged 16 months; burns on hands | 18.0 |

### ADAPTABILITY OF METHOD TO THE ESTIMATION OF PHOSPHATASE IN BILE

Several investigators have attempted to measure the activity of the enzyme in bile and have not been very successful. The new method lends itself readily to this purpose.

*Procedure.*—Dilute the bile 1:10 and 1:100 with normal saline. Treat the diluted bile as serum in the method described for serum. Two dilutions of the bile are necessary, since some biles contain enormous activity (7,000 units per 100 c.c.). A discussion of this is, however, reserved for a future paper.

SUMMARY

1. A new method is described for estimating serum phosphatase activity in which the enzyme is allowed to act on phenyl phosphate and the liberated phenol is determined.

2. The method is adaptable for use with bile.

3. The time required for duplicate analysis of a serum is about one hour.

4. The accuracy of the method is considered to be as great as or greater than that of previously described methods.

5. The procedure is simple, and only the ordinary glassware found in a clinical laboratory is required.

6. The unit by this method is almost the same numerically as that of Jenner and Kay.

REFERENCES

1. ARMSTRONG, A. R., KING, E. J. AND HARRIS, R. I.: Phosphatase in obstructive jaundice, *Canad. M. Ass. J.*, 1934, **31**: 14.
2. BENEDICT, S. R. AND THEIS, R. C.: Colorimetric determination of phenols in the blood, *J. Biol. Chem.*, 1918, **36**: 95.
3. BODANSKY, A.: Phosphatase studies; II. Determination of serum phosphatase, *J. Biol. Chem.*, 1933, **101**: 93.
4. FOLIN, O. AND CIOCALTEU, J.: Tyrosine and tryptophane determinations in proteins, *J. Biol. Chem.*, 1927, **73**: 627.
5. JENNER, H. D. AND KAY, H. D.: Plasma phosphatase: III. a clinical method for the determination of plasma phosphatase, *Brit. J. Exp. Path.*, 1932, **13**: 22.
6. KAY, H. D.: Plasma phosphatase in osteitis deformans and in other diseases of bone, *Brit. J. Exp. Path.*, 1929, **10**: 253.
7. KAY, H. D.: Plasma phosphatase: I. method of determination: some properties of the enzyme, *J. Biol. Chem.*, 1930, **89**: 248.
8. KING, E. J.: 1934 (to be published).
9. ROBERTS, W. M.: Variations in the phosphatase activity of the blood in disease, *Brit. J. Exp. Path.*, 1930, **11**: 90.
10. ROBERTS, W. M.: Blood phosphatase and the van den Bergh reaction in the differentiation of the several types of jaundice, *Brit. M. J.*, 1933, **3773**: 734.
11. ROBISON, R.: Hexosephosphoric esters in ossification, *Biochem. J.*, 1923, **17**: 286.
12. ROBISON, R., HANSMAN, F. S. AND MARTLAND, M.: Phosphoric esterase of blood, *Biochem. J.*, 1924, **5**: 1152.

# THE DIFFERENTIAL DIAGNOSIS OF COMA

BY GEORGE S. YOUNG,

*Toronto*

A GENERATION or so ago therapeutics had little to offer in cases of coma but nursing and waiting. Diagnosis, although highly desirable, was not urgently needed. Since then the discovery of insulin and the rapid development of brain surgery have introduced new methods of treatment, and early and accurate diagnosis has assumed new importance. Perhaps this may be considered sufficient excuse for reviewing the subject of the differential diagnosis of coma.

For the present purpose coma may be defined broadly as a loss of consciousness, more or less profound, transient or prolonged; excepting only the physiological condition of sleep. Whatever may be said about the nature of mental processes and mental awareness, both of these are associated with the activity of cerebral cells, and during coma the function of at least some of these cells is in abeyance. We know that this loss of function may occur from various causes.

1. *Cerebral anæmia.*—It is probable that, fundamentally, cerebral anæmia is the cause of most cases of sudden coma, since interference with cerebral circulation may be brought about in various ways, such as remote or local hæmorrhage, vasomotor disturbance, cerebral œdema, or compression from trauma. Familiar examples of the *obvious* cases of cerebral anæmia are vasovagal syncope, as in fainting, and serious external or internal hæmorrhage remote from the brain itself. To be effective in producing coma severe extracranial loss of blood must be sudden, because nature may acquire a tolerance for an extreme degree of anæmia if she has sufficient time.

2. *Mechanical injury of the brain.*—This may produce coma, even if it is so slight as to cause nothing more than concussion, with little or no pathological lesion so far as we know.

3. *Convulsive attacks.*—Coma, generally of short duration, follows epileptic seizures. It may be noted that a recent text-book has enumerated forty-eight other causes of fits beside epilepsy. The diagnosis of epilepsy without a history may therefore require a good deal of exclusion.

4. *Cerebral vascular accidents.* — Such as hæmorrhage, thrombosis and embolism.

5. *Poisons.*—(a) *Exogenous agents,* inhaled, ingested or injected. A few of these, *e.g.,* carbon monoxide, may act with great rapidity; others, as in chronic lead poisoning, may produce coma after months or years of exposure. (b) *Endogenous toxic products.*—These are conveniently subdivided into the toxæmias associated with acute infection like typhoid fever, and those cases arising from disturbed metabolism, as in