CXXXII. NORMAL VARIATIONS OF THE INORGANIC PHOSPHATE OF BLOOD.

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Consensus of experimental data, obtained from a large number of individual subjects, goes to show that the normal value for the inorganic phosphate of whole blood in man ranges from 1·9 mg. P to 3·8 mg. P per 100 cc. [de Wesselow, 1924], but singularly scant attention has been paid to the normal variations in the individual subject. One may not assume, as in the case of blood sugar, that in conditions generally considered to be normal, the inorganic phosphate remains absolutely steady, and that the taking of one sample will give a reliable figure.

In the course of experiments on the changes in blood phosphate under various physiological conditions, we investigated this question, and obtained results which it seems important to bear in mind in any similar work in the future, and also in the interpretation of some observations in the past, where the principle of ensuring a good normal does not seem to have been rigorously observed.

Method.

The method employed was a micro-adaptation of Briggs' modification of the Bell-Doisy technique [Briggs, 1922]. We consider that this adaptation, involving as it does the taking of only 0·5 cc. of blood, may have some clinical use. Moreover, in physiological experiments where a long series of samples is required, its convenience will be obvious.

Fifteen drops of blood are drawn from a pricked finger into a small crucible containing 0·5 mg. of sodium oxalate. 0·5 cc. of the blood is immediately delivered from an Ostwald pipette into a 6 cc. sampling tube containing 3·5 cc. of 0·5 % trichloroacetic acid (accurately measured). This concentration of acid has been shown to inactivate a phosphoric esterase in blood, which would otherwise give high figures for the inorganic phosphate [Martland, Hansman and Robison, 1924]. The blood is laked by inverting the tube once or twice, closed by the thumb, the proteins precipitated by the addition of 1 cc. of 22 % trichloroacetic acid, and the tube well shaken. Vigorous shaking is necessary to ensure a clear protein-free filtrate. The proteins are filtered off through a 6 cm. Whatman No. 50 filter paper, the filtrate, generally about 3 cc., being collected in a test tube. The inorganic

phosphate in the filtrate is estimated on the same day, since acid hydrolysis gives appreciable errors if analysis be further delayed.

Small tubes (9 cm. long, and 0·7 cm. in diameter), graduated to hold 1·5 cc., are used as standard "flasks."

To 1 cc. of blood filtrate are added 0·1 cc. of Briggs' molybdate reagent; 0·05 cc. of Briggs' quinol reagent; 0·05 cc. of Briggs' sulphite reagent, and the whole made up to the 1·5 cc. mark. This is done in duplicate for each blood sample.

As standard 0·5 cc. of Briggs' dilute phosphate standard (1 cc. = 0·01 mg. P) is used, with the addition of 0·2 cc. of 22 % trichloroacetic acid to ensure the same degree of acidity as in the blood filtrate.

The solutions are allowed to stand for the usual half hour and read against the standard set at 20 mm. in a microcolorimeter. We have found the Bausch and Lomb instrument to give excellent results. The average discrepancy between duplicates was under 1 %.

Normal Variations.

A large number of experiments were done in which the subject walked to the laboratory in the morning and remained seated during the experiment. Blood was immediately taken on arriving, and thereafter as a general rule at intervals of 20 minutes. It was found that the inorganic phosphate was very unsteady, varying sometimes by as much as 14 % between the taking of the first and second samples, and usually dropped about 11 %. A steadier normal had therefore to be obtained. The difficulty was solved by allowing the subject to sit at rest for about 1½ hours before taking blood, and then almost invariably the variation in blood phosphate between successive samples was found to be below 2 %, and in many cases was 0 %. The probable reason for this drop becomes evident in the light of our results on the influence of exercise on the inorganic phosphate of the blood [Havard and Reay, 1925]. We have shown that on resting after exercise a drop in the inorganic phosphate occurs to an extent which is dependent on the vigour of the exercise and on the state of training of the subject. After the mild exercise of walking, the curve reaches in about 1½ hours a flat minimum, which provides a suitable base line from which to measure the effects of any superimposed experimental condition. This minimum contrasts well with the sharp minimum followed by a rapid rise obtained after vigorous exercise.

In Fig. 1 curves *C* and *D* show the steady normal which may be obtained by the subject resting. Curve *B* shows that even if the subject only moves about doing laboratory work, the phosphate may show greater variation.

We have observed on several occasions that a definite rise has taken place in blood phosphate about midday. This increase may amount to 16 % above the morning level (Fig. 1, Curve *A*, and Fig. 2, Curve *A*). This may possibly be correlated with the afternoon rise in phosphate excretion, investigated by Fiske [1921]. On other occasions, however, this rise in blood phosphate was

absent, and in one experiment (Fig. 1, Curve *D*) a drop of 13 % was noted between 2 p.m. and 4 p.m.

Much further work is required to elucidate the cause of these variations, which probably reflect some alteration in general metabolism.

Since these irregular changes cannot be predicted, the morning seems to be the time most suitable for experiments, and results obtained in the afternoon must be interpreted with caution.

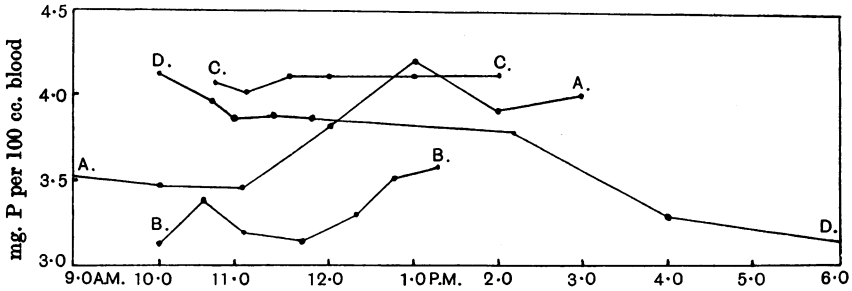

Fig. 1. A.—[R.E.H.] Rest. B.—[R.E.H.] Ordinary Laboratory work. C.—[E.W.] Rest. D.—[R.E.H.] Rest.

In order, therefore, to obtain a good normal blood phosphate for experimental work the subject should be seated in the laboratory as early in the day as possible to allow his blood phosphate to settle to a constant level and the experiment should be concluded as soon as possible in order to avoid complications due to the afternoon variations. We do not claim that a steady normal value will invariably be produced even by taking these precautions, but the value is sufficiently steady over two to three hours to make any experimental change obtained quite unmistakeable.

Influence of Sleep.

Although much work has been done on the influence of sleep on the constituents of blood and urine, it appears that only two previous observations on blood phosphate have been made, viz., by Gollwitzer-Meier and Kroetz [1924]; and by Haldane, Wigglesworth, and Woodrow [1924].

Rises in inorganic phosphate of 20 to 50 % due to sleep were noted by these workers. We have confirmed and extended these observations in the course of three experiments on one subject, R.E.H.

Exp. 1 lasted for a period of 30 hours (Fig. 2, Curve *A*), during which time the subject fasted, and was at rest either on a bed or in a camp chair, so that all exercise effects were cut out. The average day level (10 a.m.–10 p.m.) for inorganic phosphate was 3·56 mg. P per 100 cc., whilst the average sleep level was 5·06 mg. P per 100 cc., *i.e.* 42 % higher.

The sleep figure is the average of three analyses:

2.40 a.m.	...	...	5·19 mg. P %	
6.0	„	...	...	5·01 „
8.0	„	...	...	4·97 „

The inorganic phosphate therefore remains steady at the same high level during sleep.

In Exp. 2 the usual diurnal cycle was reversed, and after a sleepless night, the subject slept from 10 a.m. until 3.25 p.m. A rise of 19 % in the blood phosphate was noted during the sleep period (Fig. 2, Curve *B*).

In both these experiments the inorganic phosphate fell rapidly to the lower level after the subject awoke.

In a third experiment half an hour's sleep produced no rise in inorganic phosphate, so that probably some considerable time elapses before the sleep level is reached.

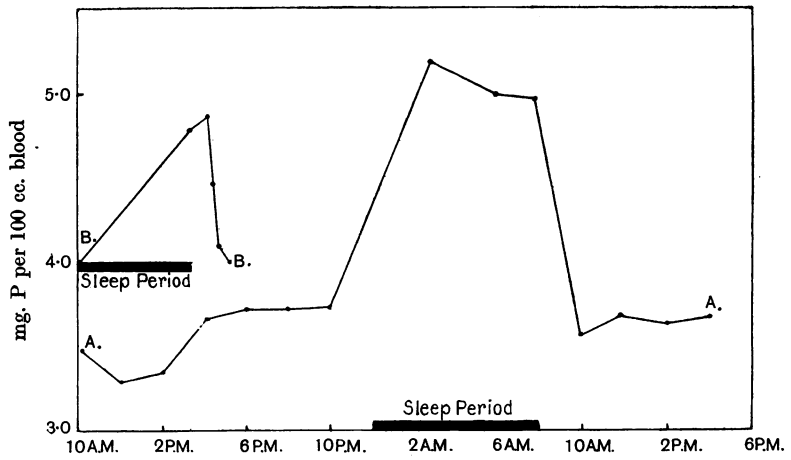

Fig. 2. Influence of sleep on inorganic P of blood.

Campbell and Webster [1922] and Kleitman [1923] have employed this reversal type of experiment in studying kidney excretion during sleep, but so far as we know it has not been carried out before for the inorganic phosphate of the blood. We have shown that the rise in inorganic phosphate of the blood cannot be separated from the phenomenon of sleep and that it is not an evidence of a diurnal physiological rhythm unconnected with sleep. This agrees with the observations by Campbell and Webster [1922] that there is an increased excretion of phosphate specifically associated with sleep. It has been shown that during sleep there is an increase in the alveolar CO_2 [Endres, 1923; Leathes, 1919] and a slightly decreased alkaline reserve and consequent increase in c_H [Collip, 1920]. These have been accounted for by assuming that the respiratory centre is less sensitive in sleep. A similar acidosis experimentally produced by Haldane, Wigglesworth and Woodrow [1924] by breathing CO_2 also caused a rise in the inorganic phosphate of the blood and an increased urinary excretion, and this has also been confirmed by us [Havard and Reay, 1925]. The evidence therefore points to CO_2 acidosis as the cause of the "sleep" rise in inorganic phosphate of the blood.

It is worthy of note that sleep is the only physiological condition associated with such a high blood phosphate in the adult. Moreover experimental CO_2 acidosis [Haldane, Wigglesworth and Woodrow, 1924] and phosphate ingestion [Wigglesworth and Woodrow, 1923] have not produced such high values as have been obtained in the case of sleep. It is true that Knipping [1922] has reported a value of 17·3 mg. P per 100 cc. of blood after "mental work." Since, however, he employed the uranium nitrate method, which gave him "normal" values as high as 8·9 mg. P per 100 cc., we do not consider these figures reliable. Moreover, in a single experiment of our own, on the influence of mental work, we could detect no change in the inorganic phosphate.

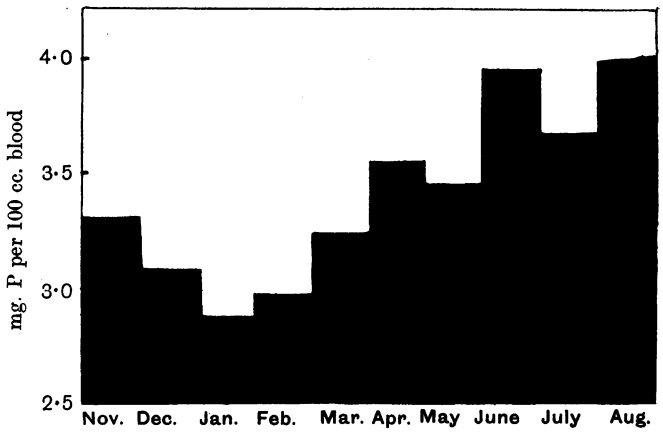

Fig. 3. Seasonal variation of inorganic P of blood.

Seasonal Variations.

Between November 1924 and August 1925 we have made some 30 determinations of the normal morning blood phosphate at rest, on various subjects and on different days. The figures obtained show a definite seasonal variation of the type noted by Hess and Gutman [1922] in children, varying from an average of 2·9 mg. P per 100 cc. blood in January to an average of 4·0 mg. P per 100 cc. blood in August (Fig. 3). The irregularity noted during May, June and July, is due no doubt to the comparatively small number of samples. Three subjects, from whom blood has been obtained both in winter and summer, all show a marked increase in the normal for the summer months. Hess and Gutman consider the effect observed in children to be due to the increased intensity of the ultra-violet rays in summer, and it is probable that this also accounts for the changes occurring in the adult. The observation is of some interest in connection with the recent work on heliotherapy.

SUMMARY.

1. A micro-adaptation of Briggs' method of blood inorganic phosphate determination is described which requires only 0·5 cc. of blood.

2. Precautions necessary in obtaining a steady "normal" blood phosphate figure are described and discussed.

3. The rise observed during sleep is confirmed and shown to occur even during sleep by day.

4. A seasonal variation is noted in the average normal blood phosphate of from 2·9 mg. P per 100 cc. in January to 4·0 mg. P per 100 cc. in August.

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