VARIATION, DIURNAL AND OVER LONGER PERIODS OF TIME, IN BLOOD HAEMOGLOBIN, HAEMATOCRIT, PLASMA PROTEIN, ERYTHROCYTE SEDIMENTATION RATE, AND BLOOD CHLORIDE

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(With 1 Figure in the Text)

Although the composition of blood is assumed to be constant within narrow limits, fluctuations in concentration of its constituents have been found by many observers over the period of a day or longer. Shaw (1927), Jores (1934) and others describe daily fluctuations in the white corpuscles, and Sabin *et al.* (1925) found similar changes in the blood platelets.

The literature dealing with diurnal fluctuations in the red blood corpuscles (R.B.C.) is already large and has been recently reviewed by Branwood (1946). Changes over longer periods of time have been described by Platt & Freeman (1930), Jellink (1936), and seasonal variations were investigated by Pucher *et al.* (1934). Diurnal and seasonal changes in the biochemical constituents have been described by some observers and denied by others.

A true diurnal rhythm should be regular and recurrent, but may, nevertheless, be modified or hidden by changes in behaviour of the individual or his environment. In order to evaluate the causes of fluctuation over a period of time, many factors must be taken into consideration. Uniformity in technique of measurement is essential and this includes as far as possible a state of uniformity of the individual.

Errors arise in all methods of measurement, not only in replicate determinations of the same sample, but also in the technique of blood sampling itself. The error of a method varies not only from one technician to another but in the same technician at different times, and a point sometimes forgotten is that diurnal or seasonal variation in laboratory temperature may, if large, become the source of a systematic error.

In the present study an attempt is made to determine whether a true diurnal rhythm (significant diurnal variation) is present for blood haemoglobin concentration (Hb), haematocrit value, plasma protein concentration, erythrocyte sedimentation rate (E.S.R.), and blood chloride concentration. Data are also presented to show variation over longer periods of time. Much of the material was not considered suitable for statistical analysis, and the data offered is mainly that dealing with repeated assays on the same individual (intra-individual variability), and with groups studied on the same day (group variability). A small amount of data obtained from sick individuals and from a few animals is given for comparison.

METHODS

The subjects used in this investigation were, unless otherwise stated, fit army personnel 20–30 years of age, on normal sedentary duties and during all periods of observation the amount of exertion was approximately the same. When blood samples were taken from 7 a.m. to 2.30 a.m. the subject had been up for an hour prior to 7 a.m. and remained up and about until the last sample had been taken. In a number of cases examinations were done on days when the subject went to bed after 10 p.m., sometimes remaining awake and sometimes being awakened for the blood sampling. Meals were taken at approximately 7.30 a.m., 1.30 p.m., 4.30 p.m. and 7.30 p.m. In order to minimize the possibility of repeated bleeding as a source of error only 1·5 c.c. was used for each sample, this being sufficient for all determinations. Venous blood was collected with minimum venous stasis (15 sec. or less) after the individual had been seated quietly for 15 min. with the arm horizontal. When observations were taken on bed patients no special precautions were taken except that the whole body and arm were horizontal for 15 min. before sampling. Heparin was used as anticoagulant as a film on the sampling tubes in the concentration of 0·15 mg. for 1·5 c.c. blood. This concentration has a negligible osmotic effect, and produces no significant difference in haematocrit reading and E.S.R. compared to the Wintrobe oxalate technique.

After thorough mixing, Wintrobe tubes were filled, stored at a temperature of $60 \pm 3°$ F. and the

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E.S.R. read off after 1 hr. All other estimations were commenced within 2 hr. of sampling. For the haematocrit estimation, tubes were spun at a speed of 3500 r.p.m. until constant packing occurred, usually in about 30 min. The supernatant plasma was pipetted off, and was sufficient for the protein estimation done by the specific gravity method of Phillips *et al.* (1945), which gives means about 0·2 g. higher than the standard micro-Kjeldahl technique. The haemoglobin was estimated on well-mixed blood by a standardized Sahli technique, using calibrated pipettes and dilution tubes, but it was not possible to calibrate the colour standard. Whole blood chlorides were done by a standard micro-method in preference to plasma chloride, partly because of the small amounts of plasma available, and partly because chloride shift is of no importance in whole blood and eliminates the necessity for blood collection under paraffin.

estimations on the same blood sample, by the same individual, for both a high and a low normal value, was determined for each variate as the standard deviation and coefficient of variation. The figures below represent the mean S.D. and C.V., and are to be accepted only as an indication of the error of methods used in this paper and independent of systematic errors. The same technicians, apparatus and methods were used throughout the whole period of study.

Variate	Standard deviation	Coefficient of variation (%)
Haemoglobin	0·26 g. %	1·65
Haematocrit	0·52 %	1·10
E.S.R.	0·75 mm.	40·00
Plasma protein	0·05 g. %	0·77
Blood chloride	0·80 mM	0·97
Clinical thermometers	0·12° F.	0·12

Table 1. *Diurnal change. Means and* S.D. *for various hours*

Variate	9 a.m.	12 noon	3 p.m.	6 p.m.
Haematocrit (%)	45·75 ± 0·45	44·99 ± 0·45	44·04 ± 0·47	43·84 ± 0·54
	S.D. ± 2·78	S.D. ± 2·81	S.D. ± 2·95	S.D. ± 3·39
E.S.R. (mm.)	2·30 ± 0·67	3·64 ± 0·67	3·76 ± 0·57	3·94 ± 0·59
	S.D. ± 4·20	S.D. ± 4·25	S.D. ± 3·62	S.D. ± 3·71
Blood chloride (mM)	80·7 ± 0·56	81·5 ± 0·53	80·2 ± 0·43	81·2 ± 0·38
	S.D. ± 3·47	S.D. ± 3·27	S.D. ± 2·65	S.D. ± 2·35

Table 2. *Diurnal change. Means and* S.D. *of diurnal range*

Haematocrit (% of mean value)	4·26 ± 0·59	S.D. ± 3·72
E.S.R. (mm. of change)	3·80 ± 0·86	S.D. ± 5·40
Blood chloride (% of mean value)	4·54 ± 0·40	S.D. ± 2·47

Since a large part of the work was done in north India (Dehra Dun) where a large diurnal and seasonal change in air temperature may occur (both of the order of 30–40° F.), haematocrit, plasma protein estimation, E.S.R. and blood chlorides were done at a temperature of 60 ± 3° F. The process of spinning blood raises its temperature by 4–5° F., but the resultant systematic error is negligible. Our own data show, however, that exposure of the same blood sample to a temperature of 104° F. for 4 hr. produces an increase in haematocrit and rise in plasma protein concentration by about 10 % compared to that done at a temperature of 68° F. Such systematic errors in haematocrit, plasma protein estimation and E.S.R. were minimized in our hands. It was, however, not possible to do the Hb estimation at a uniform temperature and it was found that a rise in laboratory temperature of about 30–40° F. could produce a systematic error of about + 0·25 g.

The error of method as given by fifteen replicate

The method of statistical analysis used in this paper is the analysis of variance (Fisher, 1941, 1942). This divides the total variation within a sample into its component parts, the significance of each component being computed from the variance ratio and its related degrees of freedom.

The nature of all investigations were explained to the subjects in the hope of minimizing changes due to emotional disturbances. There was no change in the status of nutrition or training of any individual during the periods of observation.

Wet- and dry-bulb readings were taken with a whirling hygrometer.

RESULTS

Data obtained from forty normal British individuals examined on thirty-five different days in England (August–September 1944) are shown. Table 1 gives the means, S.D. and S.E. means for the various times of the day, and Table 2 the mean, S.D. and S.E.

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mean of the diurnal range. The significance of the diurnal variation as obtained by analysis of the variance is given in Table 3. Similar results were obtained from fifteen British army personnel and fifteen Iraqui civilians in Baghdad (November–March 1943).

It is seen that the means show a progressive fall in haematocrit value, a small progressive rise in the E.S.R. and a small irregular change in the blood chloride level between the hours of 9 a.m. and 6 p.m. As may be expected the change of the variates in any individual were much more marked and could show a rise, fall or little change. The analysis of variance shows that of the three variates examined a true diurnal rhythm is present only for the haematocrit value.

In order to ascertain whether patients at complete bed rest showed diurnal variation, twenty civilian Iraqui patients were examined in Baghdad (November–March 1943). All patients had been at complete bed rest for at least a week and were not allowed up for any purpose. Data from five typical cases are given above.

The results are more or less similar to those found in the normal group.

Data obtained from ten normal rabbits examined on different days, one normal dog and a normal horse are given in Table 5. In the case of the rabbits only 0·5 c.c. of blood was used for a sample, and the animals were at complete rest and not fed during the period of observation. The dog was at rest and fed at 2 p.m. The horse remained upright, was fed at 2 p.m. and only walked a few yards during the period of observation.

Table 3. *Significance of diurnal variation from analysis of variance*

Haematocrit	$p < 5\%$ sig.
E.S.R.	Not sig.
Blood chloride	Not sig.

Table 4. *Sick individuals at complete bed rest*

Variate	7 a.m.	10 a.m.	1 p.m.	4 p.m.	7 p.m.	Diagnosis
Haematocrit (%)	34·5	35·5	34·0	34·0	28·0	Gastric carcinoma
E.S.R. (mm.)	57·0	60·0	58·0	56·0	50·0	
Haematocrit (%)	31·5	28·5	26·5	29·5	29·0	Undiagnosed fever
E.S.R. (mm.)	14·0	43·0	42·0	17·0	15·0	
Haematocrit (%)	37·5	36·0	33·0	34·0	33·0	Diabetes apyrexial
E.S.R. (mm.)	23·0	22·5	18·0	45·0	44·0	
Haematocrit (%)	39·5	38·5	—	35·0	32·0	Diabetes apyrexial
E.S.R. (mm.)	30·0	42·0	—	44·0	46·0	
Haematocrit (%)	30·5	31·5	31·5	32·5	31·0	Pyelitis pyrexial
E.S.R. (mm.)	60·0	43·0	54·0	59·0	54·0	

Table 5. *Diurnal changes in animals*

Animal	Variate	9 a.m.	12 noon	3 p.m.	6 p.m.
10 rabbits	Haematocrit (%)	$34{\cdot}50 \pm 1{\cdot}70$	$33{\cdot}35 \pm 1{\cdot}51$	$33{\cdot}25 \pm 1{\cdot}56$	$31{\cdot}50 \pm 1{\cdot}46$
		S.D. $\pm 5{\cdot}37$	S.D. $\pm 4{\cdot}78$	S.D. $\pm 4{\cdot}92$	S.D. $\pm 4{\cdot}62$
	E.S.R. (mm.)	0·55	0·35	0·85	1·0
1 horse	Haematocrit (%)	34·5	34·0	34·0	32·0
	E.S.R. (mm.)	42·0	57·0	48·0	55·0
1 dog	Haematocrit (%)	50·0	49·0	49·0	48·0
	E.S.R. (mm.)	2·0	3·0	5·0	5·0

The significance of the diurnal variation in haematocrit value was estimated for the rabbits. Analysis of variance gave $p < 0{\cdot}1\%$, showing that a highly significant change had occurred. We may therefore assume that a diurnal rhythm is present. The best-fitting straight lines for the mean haematocrit figures at the various hours has been computed for the data in Table 1 (human) and Table 5 (rabbit). The corresponding regression coefficients are $-0{\cdot}288$ and $-0{\cdot}392$, both being significant. It is of interest that calculation showed the difference between these figures to be not significant, suggesting that in the data presented rabbits behave in a way similar to humans in respect to the rate of fall in haematocrit between the hours of 9 a.m. and 6 p.m.

The E.S.R. is very slow for rabbits, and as the

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accuracy is low at this level, analysis of variance has not been attempted.

The presence of a significant diurnal variation in a group of individuals examined on different days strongly suggests that the rhythm is similar for its individuals. However, the absence of a significant group variation may be due to individuals varying among themselves in the shape of the diurnal curve. The grouping of data obtained from different days tends to obscure such individual difference which in the analysis of variance becomes included in the 'error' variance.

The bulk of the work analysed in this paper consists of (*a*) replicate data on diurnal variation from the same individual (intra-individual variability), (*b*) data obtained from groups of individuals examined on the same day (group variability), (*c*) the effect of various procedures on diurnal variation.

INTRA-INDIVIDUAL VARIABILITY

Twenty individuals, British and Indian, were studied over a period of 7 months (March–October 1945) in north India, covering the warm, hot and monsoon period, and the results from six (four British and two Indian) individuals in whom the number of days of observation was 6–8 have been analysed. The technique used was that already given, and the individuals were up and about during all periods of observation. Table 6 gives the hour to hour means for the 6–8 days, and Table 7 the significance of the hour to hour (diurnal) and day to day (seasonal) variation as obtained from the analysis of variance.

It is seen that a significant diurnal change for Hb is present in three individuals and one of doubtful significance present in a fourth. In the case of the plasma protein there is absence of a significant diurnal change between the hours of 7 a.m. and 10 p.m. in subjects 5 and 6, but present however between 7 a.m. and 2.30 a.m. in subjects 1–4. Examination of the data from the latter subjects between 7 a.m. and 10 p.m. again showed absence of significant change. It seems, therefore, highly feasible that the significant change consists of a fall after 10 p.m. and this is shown by examination of Table 6. The E.S.R. shows absence of a diurnal rhythm in four individuals, and this is doubtful in the remaining two. All four individuals in whom blood chlorides were done show absence of a significant diurnal change.

Fig. 1 shows the weighted group means for individuals 1–4 plotted together with the weighted group mean oral temperature and the mean diurnal air temperature level over the 7 months.

A seasonal rhythm is present in all subjects for Hb level, in 5/6 for plasma protein, 3/6 for the E.S.R. and 3/4 for blood chloride level. The change consists in general of an increase in concentration of all variates during the hottest months of May and June. Since the plasma protein, E.S.R. and blood chloride estimations were done at a uniform temperature, the changes found are not due to seasonal variation in laboratory temperature. The rise in Hb level is well above that due to laboratory temperature variation (see Methods). Data in Tables 13 and 14 does not suggest that haemo-concentration is playing an important part in such changes. The range of diurnal change is of the same order over the 7 months but with a distinct tendency for a larger range in the hottest months corresponding to the larger diurnal change in both oral and air temperature.

Compared to these long-term changes, the effect of menstruation and pregnancy on Hb level, plasma protein concentration, E.S.R. and blood chloride level are of interest. The pituitary-hypothalamic system may in some way be concerned in diurnal, seasonal and endocrinal rhythms.

Variation in concentration of the R.B.C. may occur over periods of days or weeks. One group of eight individuals was examined daily for eight consecutive days in November 1943 (Baghdad) and another of the same size examined twice weekly for six occasions in September 1945 (India). Samples were taken at 10 a.m. The first group showed a significant day to day variation in haematocrit ('Z' test gave $p < 1{\cdot}0\,\%$) and the second group a significant day to day change in Hb ('Z' test gave $p < 1{\cdot}0\,\%$). In neither group was the day to day change in plasma protein, E.S.R. or blood chloride significant over these short periods of time. The order of change was rather larger than that found in groups examined on the same day. The mean haematocrit values for the eight consecutive days were: 46·6, 42·7, 41·5, 42·2, 41·9, 41·4, 43·4 and 43·9 %.

GROUP VARIABILITY ON THE SAME DAY

Two groups of ten British individuals were examined four times a day on two separate days a week apart during the monsoon period in India, when both diurnal and day to day changes in meteorological conditions are small. The total variability in the samples was divided into man to man (inter-individual variation), hour to hour (diurnal variation), and 'error' variation. Table 8 gives the means for the groups at the various hours and Table 9 the significance of the variation components as computed from the analysis of variance.

The results obtained in Table 7 may throw some light on the results seen in Table 9. Of the two groups of individuals examined in conditions of similar weather, the diurnal change in Hb level was significant in the first group and of doubtful significance in the second. Table 7 suggests that only

certain individuals show a true diurnal rhythm for Hb, and since the two groups were composed of different individuals it is possible that the relative number of such 'significant' individuals may explain the results obtained. The finding of an individual diurnal rhythm (Table 7) as well as a group diurnal rhythm (Table 9), suggests that the rhythm is similar for individuals in whom it is found. That the diurnal rhythm in the R.B.C. is due mainly to endogenous factors is suggested by its presence at all times of the year, in different climates and in different races.

Absence of a significant diurnal change in the plasma protein level during waking hours corresponds to the findings in Table 7.

The significant group diurnal variation for the E.S.R. on a given day is of great interest when compared to the absence of significant diurnal change in a large group examined on different days (Table 3), and to the low order of significant change in an individual (Table 7). This strongly suggests that the diurnal rhythm for the E.S.R. is characteristic of the day and not the individual, i.e. is conferred on the individual by exogenous factors.

The inter-individual variation is significant on both days for all variates examined, showing that individuals have their own characteristic levels of Hb, plasma protein and E.S.R. Other groups of data investigated by the analysis of variance show similar findings for haematocrit ($p < 0{\cdot}1\,\%$), body

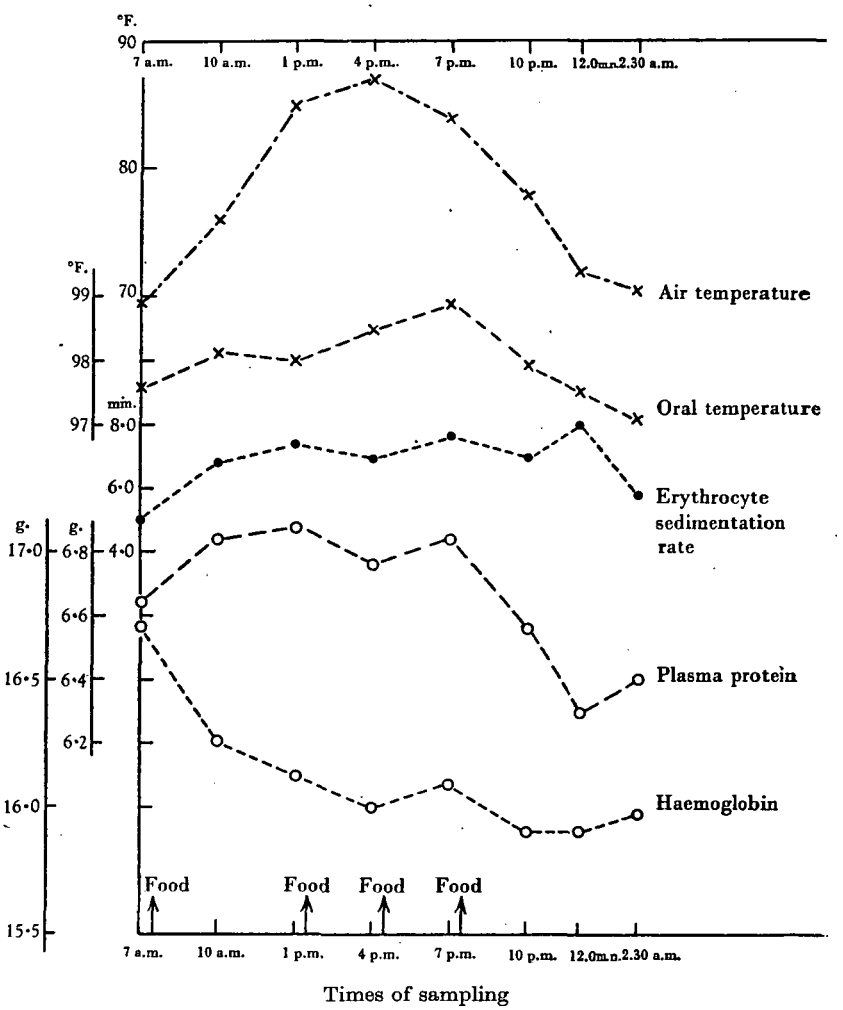

Fig. 1. Mean diurnal curves of four individuals over a period of seven months with mean diurnal air temperature over same period.

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Table 6. *Diurnal variation. Means for days at various hours*

Time ...	7 a.m.	10 a.m.	1 p.m.	4 p.m.	7 p.m.	10 p.m.	12 m.n.	2.30 a.m.	Diurnal range (% of mean)	Seasonal range (% of mean)
Subject 1, British:										
Oral temp. (° F.)	97·23	98·33	98·18	98·18	98·43	97·88	97·43	96·98	—	—
Hb (g.)	16·45	16·49	16·45	15·91	16·47	15·84	15·77	16·14	4·5	8·5
Protein (g.)	6·73	6·99	7·08	6·92	7·07	6·82	6·60	6·73	7·0	13·0
E.S.R. (mm.)	5·66	5·66	8·66	7·25	7·17	6·83	8·92	5·50	—	—
Chloride (mM)	85·25	84·25	84·75	86·00	86·50	84·25	86·25	85·25	—	—
Subject 2, British:										
Oral temp. (° F.)	97·33	98·38	98·52	98·67	98·61	98·12	97·77	97·27	—	—
Hb (g.)	16·85	16·67	16·39	16·28	16·38	16·34	16·34	16·17	4·1	11·8
Protein (g.)	6·83	6·95	7·02	6·89	6·89	6·73	6·55	6·40	9·0	3·8
E.S.R. (mm.)	0·88	1·75	1·58	2·30	2·25	2·00	2·00	2·16	—	—
Chloride (mM)	82·75	85·00	83·50	88·00	85·00	84·50	83·75	84·80	—	—
Subject 3, British:										
Oral temp. (° F.)	97·55	98·97	99·05	99·10	98·95	98·02	97·57	97·25	—	—
Hb (g.)	17·10	15·90	15·64	16·10	16·26	16·17	16·52	16·45	8·9	9·7
Protein (g.)	6·04	6·22	6·30	6·38	6·30	5·95	5·60	5·90	12·8	5·0
E.S.R. (mm.)	0·81	3·12	3·25	2·75	2·50	1·81	2·12	2·48	—	—
Chloride (mM)	86·00	87·25	85·75	86·75	86·50	85·80	85·25	86·00	—	—
Subject 4, British:										
Oral temp. (° F.)	97·08	97·98	97·90	98·18	98·42	97·75	97·48	97·20	—	—
Hb (g.)	16·38	16·05	15·91	15·70	15·28	15·20	14·93	15·12	9·0	15·3
Protein (g.)	6·96	7·17	7·17	6·89	7·10	6·89	6·50	6·54	9·7	6·8
E.S.R. (mm.)	11·38	16·38	17·50	15·50	19·25	17·25	18·62	12·35	—	—
Chloride (mM)	87·25	88·25	90·00	86·50	86·00	88·00	87·50	87·00	—	—
Subject 5, Indian:										
Oral temp. (° F.)	96·95	98·60	98·88	99·13	99·13	98·25	—	—	—	—
Hb (g.)	15·14	14·63	14·31	14·42	14·44	14·35	—	—	4·1	10·5
Protein (g.)	8·04	7·80	7·97	8·08	8·08	7·75	—	—	5·8	8·8
E.S.R. (mm.)	10·38	19·25	19·30	19·00	21·25	11·75	—	—	—	—
Subject 6, Indian:										
Oral temp. (° F.)	97·80	98·74	98·92	98·97	99·12	98·20	—	—	—	—
Hb (g.)	18·42	18·28	18·76	18·52	17·36	16·88	—	—	9·0	14·6
Protein (g.)	7·76	7·83	7·81	7·60	7·25	6·95	—	—	11·7	9·3
E.S.R. (mm.)	1·75	2·41	2·75	6·08	1·58	0·92	—	—	—	—

Table 7. *Significance of intra-individual variation obtained from analysis of variance*

	Day to day variation (seasonal)			
Subject	Hb	Protein	E.S.R.	Chloride
1	$p<0{\cdot}1\%$ sig.	$p<0{\cdot}1\%$ sig.	$p<0{\cdot}1\%$ sig.	Not sig.
2	$p<0{\cdot}1\%$ sig.	Not sig.	Not sig.	$p<0{\cdot}1\%$ sig.
3	$p<0{\cdot}1\%$ sig.	$p<0{\cdot}1\%$ sig.	Not sig.	$p<0{\cdot}1\%$ sig.
4	$p<0{\cdot}1\%$ sig.	$p<1{\cdot}0\%$ sig.	$p<0{\cdot}1\%$ sig.	$p<0{\cdot}1\%$ sig.
5	$p<0{\cdot}1\%$ sig.	$p<0{\cdot}1\%$ sig.	$p<0{\cdot}1\%$ sig.	—
6	$p<0{\cdot}1\%$ sig.	$p<5{\cdot}0\%$ sig.	$p\simeq 5{\cdot}0\%$? sig.	—
	Hour to hour variation (diurnal)			
Subject	Hb	Protein	E.S.R.	Chloride
1	$p<2{\cdot}0\%$ sig.	$p<5{\cdot}0\%$ sig.	Not sig.	Not sig.
2	Not sig.	$p<0{\cdot}1\%$ sig.	Not sig.	Not sig.
3	$p\simeq 5{\cdot}0\%$? sig.	$p<0{\cdot}1\%$ sig.	Not sig.	Not sig.
4	$p<1{\cdot}0\%$ sig.	$p<1{\cdot}0\%$ sig.	$p\simeq 5{\cdot}0\%$? sig.	Not sig.
5	Not sig.	Not sig.	$p\simeq 5{\cdot}0\%$? sig.	—
6	$p\simeq 3{\cdot}0\%$ sig.	Not sig.	Not sig.	—

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temperature ($p < 0{\cdot}1\,\%$), but not for blood chloride ($p > 10\,\%$ not sig.). Such findings throw interesting light upon the uniqueness of the individual.

THE EFFECT OF VARIOUS PROCEDURES ON DIURNAL VARIATION

The effect of prolonged exercise

These data were collected during the course of troop trials in north India. The same eight men were used on 5 days during a 20-day interval in September 1945. On the 'normal' days the men were on light sedentary duties and blood samples were taken at 9 a.m., 12 noon, 3 p.m., and 6 p.m., as already detailed, together with nude weight (bladder empty), and rectal temperature. On the exercise days, a 10-mile march was commenced immediately after the 9 a.m. sample, over a fixed route, at a uniform rate, carrying the same equipment and arriving back at the laboratory in time for the 12 noon sample. Blood samples and rectal temperatures were taken immediately on arrival followed by nude weights after drying the skin. Following rest and food, the light duties were continued and samples and observations taken at 3 p.m., and 6 p.m. as on the 'normal' days.

The severity of the exercise is shown by the mean change in weight and rectal temperature (Table 11). In Table 10 is shown the group means for the various days, and Table 11 gives the mean 9 a.m.–12 noon differences for the various days. Cursory examination does not suggest that the 3 hr. of exercise has produced obvious changes compared to the 'normal' days. However, analysis of variance shows that the variation between day to day Hb level is not significant, whilst in the case of the E.S.R. it is significant but does not correspond to the days of exercise. The data do not therefore suggest that prolonged exercise affects either the Hb level or the E.S.R. The day to day variation in the plasma protein level is on the borderline of significance, but since the variation corresponds to the days of exercise it is reasonably certain that the significance can be accepted. The following experiment was made to determine the effect of severe exertion over a short period of time. Eight men had two resting samples taken at intervals of 10 min. followed by a final sample on cessation of 10 min. of violent exertion sufficient to produce dyspnoea and marked sweating.

The means of the resting samples did not differ significantly, and the average of the means was taken as the mean resting level. Resting samples were taken to minimize variation over a period of 10 min. The results are summarized in Table 12.

Table 8. *Diurnal variation. Group means for the same day at different hours*

Group 1. Means for ten individuals

Time ...	9 a.m.	12 noon	3 p.m.	6 p.m.	Diurnal range (% of mean)
Dry-wet bulb (° F.)	79–76	80–76	80–76	80–76	
Hb (g.)	15·63	14·78	14·39	15·16	8·27
Protein (g.)	6·53	6·47	6·42	6·38	2·17
E.S.R. (mm.)	7·67	8·89	12·17	11·72	—

Group 2. Means for ten individuals

Time ...	9 a.m.	12 noon	3 p.m.	6 p.m.	Diurnal range (% of mean)
Dry-wet bulb (° F.)	83–75	85–78	85–78	86–79	
Hb (g.)	15·37	15·13	14·88	15·12	3·2
Protein (g.)	6·79	7·02	6·92	6·87	3·3
E.S.R. (mm.)	8·17	11·63	11·06	14·72	—

Table 9. *Significance of group variation obtained from analysis of variance*

	Group	Hb	Plasma protein	E.S.R.
Diurnal variation	1	$p < 0{\cdot}1\,\%$ sig.	Not sig.	$p < 1{\cdot}0\,\%$ sig.
	2	$p > 5\,\%$? sig.	Not sig.	$p < 0{\cdot}1\,\%$ sig.
Inter-individual variation	1	$p < 0{\cdot}1\,\%$ sig.	$p < 0{\cdot}1\,\%$ sig.	$p < 0{\cdot}1\,\%$ sig.
	2	$p < 0{\cdot}1\,\%$ sig.	$p < 5\,\%$ sig.	$p < 0{\cdot}1\,\%$ sig.

Most observers find a rise in Hb and plasma protein level with both short and long bouts of exercise, but the time factor has not been sufficiently considered. The lack of rise in Hb concentration with prolonged exercise (Table 11) corresponds to the findings of Hawk (1904), Broun (1922), and Davis & Brewer (1935), but it is reasonable to assume that the diurnal fall in Hb is playing some part. The doubtful significant effect of exercise on the E.S.R. corresponds to the results found by Rourke & Plass (1929).

The effect of changes in water balance on diurnal variation

In order to assess the effects of change in water balance over the period of 9 a.m. to 6 p.m., experiments were done on three subjects (nos. 1, 2 and 4 of Table 6) using as control data obtained from the same individuals whilst on normal water balance during the same period of the year and in the same laboratory.

Table 13 gives the data from subject no. 2 obtained in England during August 1944, and that of Table 14

Table 10. *Group means for various days*

Time ...	9 a.m.	12 noon	3 p.m.	6 p.m.	
Dry-wet bulb (° F.)	80–77	80–76	80–76	80–76	1. 'Normal' day
Hb (g.)	15·96	15·10	14·66	15·26	
Protein (g.)	6·65	6·60	6·61	6·61	
E.S.R. (mm.)	7·4	9·3	15·0	14·5	
Dry-wet bulb (° F.)	77–75	78–77	78–77	81–78	2. Exercise day
Hb (g.)	15·41	15·31	14·88	15·13	
Protein (g.)	6·69	6·99	6·78	6·9	
E.S.R. (mm.)	9·5	6·5	9·2	9·2	
Dry-wet bulb (° F.)	79–75	81–76	82–78	81–77	3. Exercise day
Hb (g.)	15·12	14·84	14·98	15·05	
Protein (g.)	6·80	6·97	6·73	6·85	
E.S.R. (mm.)	7·2	9·3	11·5	11·9	
Dry-wet bulb (° F.)	78–76	83–77	84–77	84–77	4. Exercise day
Hb (g.)	14·98	15·21	14·54	14·84	
Protein (g.)	6·77	7·03	7·03	6·98	
E.S.R. (mm.)	9·3	12·6	13·5	10·7	
Dry-wet bulb (° F.)	84–75	88–78	87–78	86–79	5. 'Normal' day
Hb (g.)	14·97	14·79	14·56	14·84	
Protein (g.)	6·91	6·97	6·95	6·82	
E.S.R. (mm.)	8·6	12·0	11·75	16·0	

Table 11. *Mean differences between 9 a.m. and 12 noon samples*

	Hb (g.)	Plasma protein (g.)	E.S.R. (mm.)	Weight (lb.)	Rectal temp. (° F.)
1. 'Normal' day	−0·86	−0·05	+1·9	+0·2	−0·59
2. Exercise day	−0·10	+0·30	−3·0	−3·5	+0·95
3. Exercise day	−0·28	+0·17	+2·1	−3·4	+1·0
4. Exercise day	+0·23	+0·26	+3·3	−4·4	+1·65
5. 'Normal' day	−0·18	+0·06	+3·4	−0·1	+0·02
Day-to-day variation from 'Z' test	Not sig.	$p \simeq 5\%$? sig.	$p < 0{\cdot}1\%$ sig.	$p < 0{\cdot}1\%$ sig.	$p < 0{\cdot}1\%$ sig.

Table 12

Variate	Mean difference exercise-resting	S.E. difference	Significance
Hb (g.)	+0·42	0·91	$p < 5\%$ sig.
Protein (g.)	+0·34	0·13	$p < 10\%$? sig.
E.S.R. (mm.)	+2·06	1·17	$p < 20\%$? sig.

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from subjects nos. 1 and 4 during the hot month of June in north India. The legends give details of the experimental procedure.

Examination of Table 13 suggests that the experimental days have produced a fall in Hb level, but the amount of fall lies within the range of variation seen in the 'normal' days. We may have here an explanation of the controversial statements in the literature as to the effect of pituitrin and water on plasma protein during the summer months is not an index of haemo-concentration, but rather a change in the 'normal' level.

It is of course possible that the experimental days have produced a real change, which might become obvious from replication of 'normal' and experimental days with an analysis of the variance. However, it is clear that great care must be taken in drawing conclusions from single experiments.

Table 13. *Diurnal changes on normal and experimental days. Subject no.* 2

Time ...	9 a.m.	12 noon	3 p.m.	6 p.m.	Range (% mean)	
Haematocrit	46·0	44·0	40·0	43·0	14·0	1. 'Normal' day
E.S.R. (mm.)	1·0	1·0	3·0	1·0		
Chloride (mM)	87·5	80·0	82·0	80·0		
Haematocrit	48·5	47·0	45·5	44·0	9·8	2. 'Normal' day
E.S.R. (mm.)	0·5	1·0	2·0	2·0		
Chloride (mM)	77·0	80·0	80·0	80·5		
Haematocrit	48·5	48·5	44·5	44·5	8·6	3. Water day. 1 l. of water per hour from 9 a.m. to 6 p.m. commencing immediately after 9 a.m. specimen. Food normal
E.S.R. (mm.)	1·5	1·5	0·5	1·5		
Chloride (mM)	76·0	80·0	78·0	78·5		
Haematocrit	47·5	46·5	44·0	41·5	13·3	4. Salt day. Approximately 5 g. per hour commencing immediately after 9 a.m. specimen (45 g. total). Water *ad lib.* Food normal
E.S.R. (mm.)	0·5	1·5	2·0	1·5		
Chloride (mM)	78·0	80·0	84·7	87·7		
Haematocrit	47·5	47·0	44·0	44·5	7·6	5. Pituitrin and water. 5 l. water between 9.30 a.m. and 6 p.m. 0·5 c.c. pituitrin at 10 a.m. and 2 p.m. No diuresis between 10.30 a.m. and 4 p.m. Food normal
E.S.R. (mm.)	1·5	0·5	1·5	2·0		
Chloride (mM)	78·7	84·5	80·0	82·0		
Haematocrit	48·0	45·5	42·0	42·5	13·4	6. Pituitrin water and salt. As above with salt as on salt day. Food normal
E.S.R. (mm.)	1·5	1·0	1·0	1·0		
Chloride (mM)	87·3	88·0	92·0	95·7		
Haematocrit	48·0	49·5	48·0	49·5	3·0	7. 'Normal' day
E.S.R. (mm.)	0·5	0·5	0·5	0·5		
Chloride (mM)	82·0	82·7	82·0	83·5		
Haematocrit	50·0	47·0	46·0	48·5	8·3	8. 'Normal' day
E.S.R. (mm.)	1·0	1·5	3·0	0·5		
Chloride (mM)	80·0	82·7	80·0	80·0		

blood concentration. On the dry day (Table 14) we have insufficient evidence for haemo-concentration in spite of a weight loss of 4·5 lb. in both subjects. During the 'water' days, in spite of a marked increase in weight (3·25 and 7 lb.), there is again no definite evidence from the data that the increased fluid intake has had an obvious effect on the Hb or plasma protein level. The data in Table 14 fit in with the view that the higher levels of both Hb and

The only change which is almost definitely significant is the effect on the blood chloride level of pituitrin salt and water in England and the effect of salt feeding on a hot day in India. In both cases the blood chloride rises to supernormal levels (maximum normal 91·4 mM). Salt feeding alone does not produce this effect in a cool climate and the changes seen in India suggest the increased production of anti-diuretic-like substances in a hot environment.

Table 14. *Diurnal changes on 'normal' and experimental days*

	Time ...	9 a.m.	11 a.m.	1 p.m.	3 p.m.	5.30 p.m.	Range (% mean)
			Subject 1				
'Normal' day	Dry-wet bulb (° F.)	90–72	98–77	104–77	106–77	106–72	
	Oral temp. (° F.)	98·0	98·1	98·0	98·4	98·4	
	Hb (g.)	17·50	16·80	16·80	16·80	16·50	5·97
	Protein (g.)	7·35	7·55	7·20	7·55	7·55	
	E.S.R. (mm.)	11·0	13·0	15·0	15·0	12·5	
	Chloride (mM)	87·0	87·7	83·5	—	87·7	
	Body wt. (lb.)	140·0	139·5	139·25	140·0	139·25	
Dry day	Dry-wet bulb (° F.)	90–71	95–77	100–77	103–77	100–78	
	Oral temp. (° F.)	97·9	98·2	99·0	99·0	99·0	
	Hb (g.)	16·50	15·40	16·00	16·50	16·50	5·5
	Protein (g.)	7·70	7·35	7·70	7·70	7·70	
	E.S.R. (mm.)	11·0	11·0	15·0	17·5	15·0	
	Chloride (mM)	82·7	87·0	81·0	89·7	81·7	
	Body wt. (lb.)	138·5	138·75	137·5	136·5	134·5	
Water day	Dry-wet bulb (° F.)	84–75	92–78	98–78	100–76	92–78	
	Oral temp. (° F.)	98·1	97·6	97·9	97·4	97·6	
	Hb (g.)	17·50	17·08	16·80	16·80	17·08	4·1
	Protein (g.)	7·55	7·20	7·00	7·20	7·55	
	E.S.R. (mm.)	4·5	3·5	8·0	3·5	7·0	
	Chloride (mM)	—	—	—	—	—	
	Body wt. (lb.)	139·75	141·5	140·5	142·25	143·0	
Salt day	Dry-wet bulb (° F.)	88–78	93–80	98–80	95–80	95–80	
	Oral temp. (° F.)	98·2	98·4	97·6	97·8	98·0	
	Hb (g.)	17·08	16·80	16·80	16·80	15·68	8·4
	Protein (g.)	7·50	7·30	7·50	7·30	7·50	
	E.S.R. (mm.)	5·0	7·0	12·0	9·0	9·5	
	Chloride (mM)	89·0	93·0	92·5	90·5	94·0	
	Body wt. (lb.)	140·0	141·75	142·0	143·75	143·5	
			Subject 4				
'Normal' day	Dry-wet bulb (° F.)	90–72	98–77	94–77	106–77	106–72	
	Oral temp. (° F.)	98·7	98·4	98·4	98·4	98·7	
	Hb (g.)	17·50	16·10	16·10	15·40	15·68	13·0
	Protein (g.)	7·85	7·85	7·50	7·50	7·20	
	E.S.R. (mm.)	11·0	21·0	23·0	16·0	20·0	
	Chloride (mM)	86·5	89·0	86·5	84·7	87·5	
	Body wt. (lb.)	147·0	146·25	146·5	146·75	145·75	
Dry day	Dry-wet bulb (° F.)	90–71	95–77	100–77	103–77	100–78	
	Oral temp. (° F.)	98·4	98·4	99·0	99·2	98·9	
	Hb (g.)	15·68	16·52	16·1	16·1	16·52	5·1
	Protein (g.)	7·20	7·20	7·20	7·20	7·20	
	E.S.R. (mm.)	16·0	15·0	19·0	21·0	19·0	
	Chloride (mM)	83·5	85·5	85·5	—	83·5	
	Body wt. (lb.)	146·5	145·75	144·5	143·5	142·0	
Water day	Dry-wet bulb (° F.)	84–75	92–78	98–78	100–76	92–78	
	Oral temp. (° F.)	97·6	97·8	98·4	97·6	98·2	
	Hb (g.)	16·80	16·38	16·10	14·80	14·80	12·8
	Protein (g.)	7·50	7·00	7·80	7·00	6·90	
	E.S.R. (mm.)	12·0	12·0	19·0	17·0	22·0	
	Chloride (mM)	—	—	—	—	—	
	Body wt. (lb.)	147·0	149·0	152·5	154·0	154·0	

Table 14 (*continued*)

	Time ...	9 a.m.	11 a.m.	1 p.m.	3 p.m.	5.30 p.m.	Change (% mean)
Salt day	Dry-wet bulb (° F.)	88–78	93–80	98–80	95–80	95–80	
	Oral temp. (° F.)	98·2	98·4	98·4	98·4	98·2	
	Hb (g.)	17·08	16·52	16·10	16·10	15·68	8·6
	Protein (g.)	7·00	7·50	7·20	7·50	7·20	
	E.S.R. (mm.)	16·0	18·0	16·0	15·0	18·0	
	Chloride (mM)	85·7	90·0	93·5	94·0	95·7	
	Body wt. (lb.)	145·75	146·75	147·5	149·5	149·75	

Dry day. Absolute minimum water intake from 9.30 a.m. to 5.30 p.m. Food normal.
Wet day. 2·0 l. water per hour from 9.30 a.m. to 5.30 p.m. Food normal.
Salt day. 40 g. salt between 9.30 a.m. and 5.30 p.m. Water *ad lib.* Food normal.

DISCUSSION

Although the literature dealing with diurnal change in the Hb level is large, there has been little attempt to analyse statistically the data so obtained. Mole (1945), using the analysis of variance on the data of McCarthy & Van Slyke (1939), found a true diurnal rhythm to be present between the hours of 9 a.m. and 11 p.m. The error of method is relatively large for the Sahli method (S.D. $\pm$ 0·260 g.) and this must be taken into account in estimating the amount of real change in our data. With this reservation the shape of the diurnal curve (Fig. 1) and the range of variation would correspond to that found by the above authors using a refined technique.

In the present data four out of six individuals showed a significant diurnal change (Table 7), and the same was found for one group of individuals studied on the same day (Table 9). A significant group rhythm may depend upon the relative number of individuals in the group showing the same form of rhythm. Where a rhythm is absent the diurnal curve is variable, and its behaviour on a given day may be unpredictable. It would appear that the haematocrit behaves in a similar manner to the Hb level. However these two variables do not always run parallel, since the R.B.C. may swell independently of the R.B.C. concentration. The finding of a diurnal rhythm in the R.B.C. in several races, in sick individuals (Table 4) and in a few animals (Table 5) suggests that some biological basis may be present.

In the case of the plasma protein level it has been shown that significant changes are absent during normal waking hours of 7 a.m. to 10 p.m. with a significant fall after 10 p.m., independent of bed rest, with the suggestion of a rise commencing about 2.30 a.m. (Fig. 1). The minimum concentration after 10 p.m. in any individual was 5·50 g. and a larger series might have shown even lower figures. Dyson & Plaut (1943), using the micro-Kjeldahl technique, found small changes, but larger than the error of method to occur between the hours of 9 a.m. and 6 p.m. Perera & Berliner (1943), using a specific gravity method, found changes during waking hours of a similar order to that found by us.

Posture undoubtedly affects the concentration of the R.B.C. and plasma protein, but the subjects in this paper were up and about during the whole period of observation. Two individuals spent 1 day in bed, rose at 7 p.m. and remained up till 2.30 a.m. On the next day they remained up during the day and retired to bed at 10.30 p.m. The changes in oral temperature, Hb, and plasma protein level were very similar on both days with the nocturnal fall being a little more marked for plasma protein during bed rest. A progressive fall in plasma protein during bed rest in waking hours was however absent. Sleep itself seems to have no obvious effect on any of the blood variables.

In our hands horizontal rest for a period of 30 min. produced a mean significant fall in plasma protein level of 0·35 g. ($p < 0·1$ %), but as shown in Table 6 a rise or fall may occur during waking hours without any obvious change in posture. In an experiment designed to eliminate variation due to time, prolonged bed rest of 48 hr. in normal individuals produced a significant mean fall of 0·65 g. ($p = 1·0$ %) in the 10 a.m. specimen. This result may be due to factors other than posture, and is paralleled by the fall in mean and range of body temperature on prolonged bed rest. Assuming 5·80 g. to be the minimum concentration during waking hours, prolonged bed rest *per se* in a normal individual may lower this to 5·0–5·20 g. a figure generally accepted as representing hypoproteinaemia. This fact must be taken into consideration in studies dealing with malnutrition, and may throw light on the low correlation found between plasma protein level and oedema (Mollison, 1946; Mitchell & Black, 1946; and others). Authors sometimes do not mention the fact that an initial estimation is done with the patient in bed and another after the patient has been up and about for a few days.

The cause of the rhythm in Hb and plasma protein concentration is far from clear. It has been shown that moderate changes in water balance (Tables 13 and 14) may have little obvious effect on either the Hb or plasma protein level, and it is unlikely that normal water intake is of great importance.

The relation of the diurnal rhythm to food intake (Fig. 1) does not suggest that it is due to this factor, and in any case one would expect a post-prandial haemo-concentration. The dissociation between the Hb and plasma protein levels during waking hours is not likely to be due to technical factors, and fits in with the low order of correlation between these variables found in these data ($r = 0{\cdot}150$ sig.). Menzel (1940) and Gollwitzer-Meier (1931) found an increase in plasma volume to occur at night independently of sleep, fluid intake and posture, and this associated with a fall in the dried solids of the plasma. The results found in Table 11 do not suggest that the ordinary activities of sedentary life play much part in the diurnal changes. It is therefore possible that rhythmic changes in liberation, distribution or removal of red cells during the day, and increase in the plasma water at night may be factors to consider. The hypothalamic-pituitary system may be concerned in such changes. Although exertion, posture, fluid, food and emotional factors are not the cause of the rhythm, they may modify it, especially in certain individuals.

The results for the E.S.R. suggest that neither an individual nor a group of individuals examined on different days have a diurnal rhythm. The presence of a group rhythm on a particular day (Table 9) is hence due to the conditions of that day and probably related to meteorological or other cosmic factors. Jores & Strutz (1936) came to a similar conclusion for the diurnal changes in E.S.R., and Hoverson & Peterson (1934) suggested that the day to day changes are due to similar causes.

The diurnal changes in whole-blood chloride level are completely haphazard in both individuals (Table 7) and in groups of individuals (Table 3). In any individual the change may be irregular or consist occasionally of a rise or fall. This may explain the inconsistency found in the literature dealing with changes in the whole-blood chloride during the day and night (Jores, 1934; Gollwitzer-Meier & Kroetz, 1924). It is unlikely that the diurnal changes are related to changes in the R.B.C., and scatter diagrams suggest a low order of correlation between the diurnal haematocrit or Hb level and the diurnal whole-blood chloride in an individual or a group. If the plasma chlorides remained unchanged during the day, one would expect a progressive rise in the whole-blood chloride level as the Hb or haematocrit fell. There is little evidence in these data for a post-prandial fall in the whole-blood chloride.

Examination of Fig. 1 suggests that a possible relationship exists between the mean diurnal change in air temperature, oral temperature, E.S.R. and plasma protein level. It is of interest that a well-marked correlation does in fact exist between the oral and air temperature in the present data ($r = 0{\cdot}673$ sig. for oral and dry-bulb temperature). However, scatter diagrams suggest little correlation between any of the variables—Hb, plasma protein, E.S.R., blood chloride, or between any of these and oral temperature.

For any of the variates examined, the variability as measured by the coefficient of variation differs markedly between individuals over the same period of time. Furthermore, variability of one variate is not necessarily correlated with the variability of any other variate. Following the method of Hammett (1920), an attempt was made to correlate the variability of individuals with a simple assessment of emotional stability. The results suggest that this line of approach is worthy of further study. Goldstein (1935) and others found a greater intra-individual variability in blood chemistry in groups of schizophrenics than in groups of normals.

The knowledge that the human shows variation in the concentration of the blood constituents is of practical as well as of theoretical interest, and if time variation is disregarded, the random error of estimation of Hb, haematocrit, plasma protein and blood chloride become very appreciable. Many experimental procedures dealing with problems of water or salt balance use these variates as indices of change occurring in the blood. Unless the variation due to time itself is known, wrong conclusions may be drawn from correct data. This is demonstrated by the findings in Tables 13 and 14. To some extent the same is true for bed patients, and here the question of posture is of importance especially in reference to the plasma protein level. Since an individual may behave differently to a group, care must be taken in drawing conclusions from an experiment using only one subject. Again, similarity in behaviour of the blood of a group of individuals examined over the same period of time, even if statistically significant, may in part or whole be independent of an experiment. This is true for periods lasting a few hours, days or months. To eliminate the time factor random controls must be used and these examined under identical conditions of time and physiological state as the experimental subjects.

It is important for the clinician to realize that in normal individuals changes up to 10 % or more may occur in the R.B.C. and plasma protein level due simply to the passage of a few hours, and prolonged bed rest may *per se* lower the plasma protein to levels generally considered pathological. Changes up to 7·0 mM (40·0 mg. %) may occur during a day in the whole-blood chloride level. An E.S.R. of 2–6 mm. in the morning may rise to 10–15 mm. by the afternoon or evening in perfectly normal individuals.

Seasonal and short term variation must be considered by workers concerned in problems of growth and nutrition.

CONCLUSIONS

The method of variance analysis has been used to determine the significance of changes occurring over various periods of time for a number of blood variates.

A significant diurnal rhythm is present for most individuals for Hb, haematocrit, and plasma protein level, but absent for the E.S.R. and blood chloride. The change in the first consists of a progressive fall from 7 a.m. to 2.30 a.m., and in the second of a fall after 10 p.m. Such changes may be affected by, but are not due to, posture, sleep, exertion, fluid or food intake. The diurnal E.S.R. curve varies from day to day, but is significantly variable on a given day, and probably related to weather changes.

A significant seasonal variation is present in most individuals for the above-mentioned blood variates and consists of higher values during a hot season.

Before conclusions can be drawn from the results of an experiment, the variations due to the passage of time must be known, and this requires design of experiment.

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