The age of the patient, however, did influence the results obtained with the administration of estrogen. In the premenopausal patients there was a daily insulin saving of 63% represented by a decrease in the daily average insulin requirement from 48 units to 18 units. In the postmenopausal patients, on the other hand, the "pre-estrogen" requirement of 108 units daily fell only 41% to 63 units daily during the administration of estrogen.

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Diurnal Variations in the Acid-Base Balance.

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Interpretation of abnormal variation in the acid-base balance of the blood presupposes a definition of what constitutes normal variations. There has been accumulated in the past a large amount of data on the pH, the serum bicarbonate concentration and the CO_2 tension of the blood of normal individuals. It is generally regarded that if, in arterial blood, the pH is between 7.35 and 7.45, the bicarbonate between 24.5 and 27.5 mM per liter, and the CO_2 tension between 40 and 45 mm, a normal acid-base balance of the blood is indicated.

Shock and Hastings,[1] in a series of 131 determinations on 57 normal individuals, found that the variation from individual to individual was no greater than the variation found in a single individual on repeated observations. Whether or not the acid-base balance of the blood of a single individual varies in any consistent manner during the course of a day is still an open question.

Cullen and Earle[2] made 5 or 6 determinations of the acid-base balance of the blood on 10 individuals between 9 in the morning and 9 at night. They came to the conclusion at that time that there tended to be an alkaline shift during the day, although this was not an invariable finding. Shock and Hastings made hourly observations on each of 10 subjects for a total of 22 experiments between the hours of 9 and 6. These individuals were permitted to go about their usual activities. Some shifts in CO_2 tension, in pH and bicarbonate were

1 Shock, N. W., and Hastings, A. B., *J. Biol. Chem.*, 1934, **104**, 585.

2 Cullen, G. E., and Earle, I. P., *J. Biol. Chem.*, 1929, **83**, 545.

observed, but the changes were not consistently in any one direction. In view of those results and the great sensitivity of the acid-base balance of the blood to slight changes in respiratory activity, it was concluded that the variations encountered in single individuals during the course of a day of normal activity was largely a matter of chance.

However, whether or not variations of a consistent nature occur when a normal subject is at bed rest during a complete 24-hour period has not previously been studied. The present paper has, for its purpose, the presentation of the acid-base changes in the blood under such conditions.

Experimental. The subject was a normal individual convalescing from a minor surgical operation. Two 24-hour experiments were completed—the first, 6 days after the operation; the second, a week later. Samples of cutaneous blood, which closely approximates arterial blood in composition, obtained by the technic described by Shock and Hastings,[3] were collected at approximately 2-hour intervals throughout the 24-hour period. The 2 a. m. sample was omitted in both experiments, the 6. a. m. sample in the first experiment.

The blood was analyzed in duplicate for the percentage cells, pH_s, and total CO_2, using the micro acid-base method described by Shock and Hastings. The duplicate CO_2 analyses agreed within 0.6 mM/liter, the pH determinations within .01. From these data, the bicarbonate concentration of the serum $(HCO_3)_s$ and the CO_2 tension of the blood (pCO_2) were calculated. By estimating what the serum bicarbonate concentrations would have been at pH 7.40 [designated as $(HCO_3)'_s$ and calculated from the equation, $(HCO_3)'_s = (HCO_3)_s + 30\ (pH—7.40)$] it was possible to determine whether any trends toward net base excess or net acid excess occurred during the 24-hour cycle. These data are given in Table I.

Comment. In experiment 1, the pH varied from 7.31 to 7.43; the $(HCO_3)_s$ from 24.1 to 28.0 mM per liter; the $(HCO_3)'_s$ from 24.2 to 28.9 mM per liter; and the pCO_2 from 38.3 to 55.2 mm Hg. In experiment 2, the pH variation was from 7.36 to 7.42; the $(HCO_3)_s$ from 26.4 to 30.3; the $(HCO_3)'_s$ from 25.2 to 29.7; the pCO_2 from 45.2 to 53.8. Such variations as these are as great as one would encounter in a series of single determinations of a large number of normal individuals. It was found that the variations were not random, however, but represented trends of a definite nature, in excess of the errors of observation.

These trends have been shown in Fig. 1 by plotting against time,

[3] Shock, N. W., and Hastings, A. B., *J. Biol. Chem.*, 1934, **104**, 565.

TABLE I.

Determination of Percentage Red Cells, V_c; pH_s; CO_2 Content of Blood $(CO_2)_b$, During 24-hour Period. Subject at Rest in Bed. Food at 8:30 a.m., 12:30 p.m., 5:30 p.m.; Sleep, 10:30 p.m. to 8:00 a.m.

	Experiment 1						Experiment 2					
	Determined			Calculated			Determined			Calculated		
Time	V_c	pH_s	$(CO_2)_b$ mM/L	$(HCO_3)_s$ mM/L	$(HCO_3)'_s$ mM/L	pCO_2 mm Hg	V_c	pH_s	$(CO_2)_b$ mM/L	$(HCO_3)_s$ mM/L	$(HCO_3)'_s$ mM/L	pCO_2 mm Hg
a.m.												
8	.44	7.42	20.7	24.1	24.7	38.3	.48	7.38	23.4	27.6	27.0	48.1
10	.44	7.41	22.9	26.7	27.0	43.4	.47	7.40	24.0	28.4	28.4	47.3
12	.49	7.43	22.6	27.2	28.1	42.3	.47	7.41	24.2	28.6	28.9	46.6
p.m.												
2	.43	7.43	24.0	28.0	28.9	43.5	.47	7.40	23.4	27.6	27.6	46.0
4	.44	7.41	23.9	27.8	28.1	45.3	.44	7.42	24.4	28.4	29.0	45.2
6	.44	7.41	21.7	25.2	25.5	41.1	.45	7.36	22.8	26.4	25.2	48.1
8	.40	7.42	22.9	26.2	26.8	41.7	.46	7.39	22.8	26.7	26.4	45.5
10	.43	7.40	21.8	25.1	25.1	41.8	.46	7.38	24.3	28.4	27.8	49.5
12	.44	7.40	22.1	25.7	25.7	42.7	.45	7.36	25.5	29.5	28.3	53.8
a.m.												
4	.44	7.31	23.8	26.9	24.2	55.2	.46	7.37	24.8	28.9	28.0	51.8
6	—	—	—	—	—	—	.46	7.38	24.4	28.5	27.9	49.8
8	.45	7.35	22.7	26.0	24.5	48.5	.46	7.38	25.9	30.3	29.7	52.8
Mean		7.40	22.6	26.3	26.2	44.0		7.39	24.2	28.3	27.9	48.7
Min.		7.31	20.7	24.1	24.2	38.3		7.36	22.8	26.4	25.2	45.2
Max.		7.43	23.9	28.0	28.9	55.2		7.42	25.9	30.3	29.7	53.8

the deviation from the mean value for the 24-hour period of the CO_2 tension (pCO_2), and of the bicarbonate concentration at pH 7.40, $(HCO_3)'_s$. The experimental data are shown by points—the mean values for the two experiments by solid lines. Considering the small

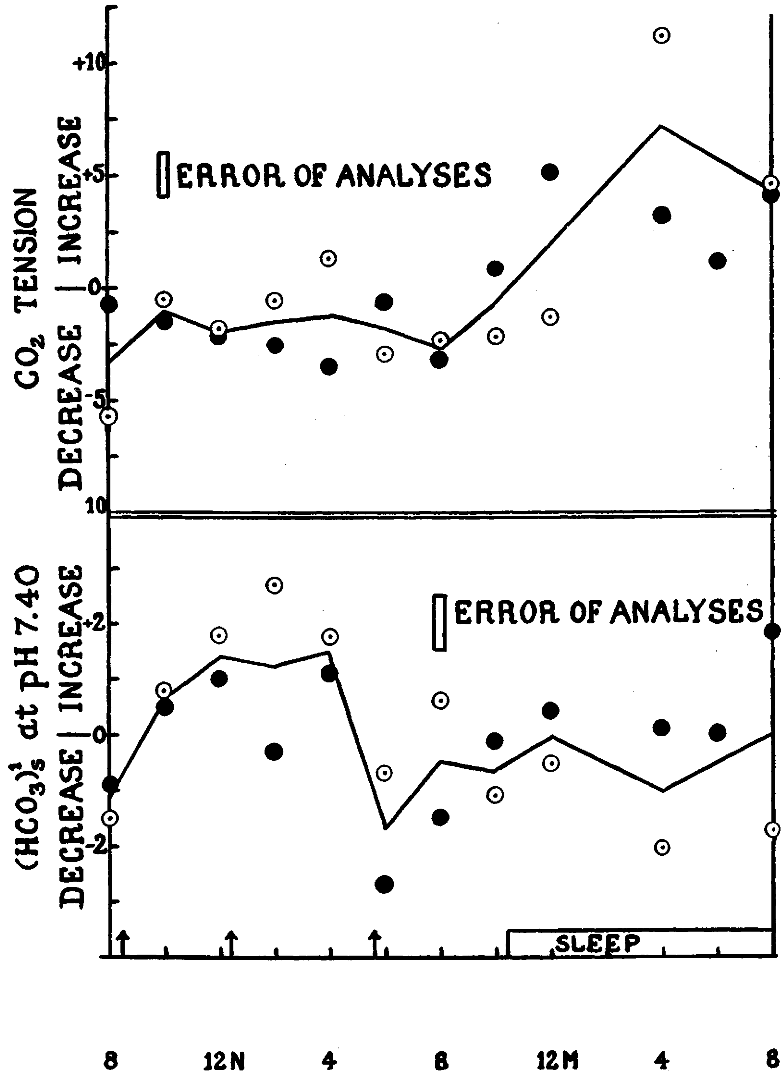

FIG. 1.

The upper chart shows the changes in pCO_2 with time; the lower chart, the changes in $(HCO_3)'_s$ with time. The data of experiment (1) are designated as ⊙; of experiment (2), as ●. The solid lines indicate the mean data of both experiments. The extent of the maximum analytical errors on pCO_2 and $(HCO_3)'_s$ are indicated by the open rectangles. The arrows indicate the ingestion of food.

magnitude of the changes involved, the two experiments are in agreement.

The data showed certain trends in the blood during the day which seem quite definite: (1) The value of $(HCO_3)'_s$ rose about 2 millimols during the forenoon and descended to its original value in the afternoon. In one experiment, the $(HCO_3)'_s$ rose again during the night; in the other, it did not. In 5 out of 6 samples taken from 1.5 to 2 hours after eating, there was an increase in $(HCO_3)'_s$ amounting to approximately 1 mM. This parameter of the acid-base balance, $(HCO_3)'_s$, is determined by the net difference between the equivalent concentrations of basic ions and acid ions (other than HCO_3) at pH 7.40. Changes in $(HCO_3)'_s$ may result, therefore, from a departure in the balance between production or absorption, and destruction or excretion of the acidic and basic components of the blood. Since physical activity was absent as a disturbing factor in these observations, it is reasonable to assume that the trend toward alkali increase after meals was due to the combined effect of the process of digestion and the acid-base balance of the food ingested.

(2) The CO_2 tension showed little variation during the day until the subject went to sleep. There was then a rise amounting to approximately 7 mm of Hg over the average CO_2 tension when awake. Indeed, the analyses made in the first experiment on blood drawn at 4 a. m. showed a rise of 11 mm above the mean value for the day. This is in agreement with the observations of Endres,[4] who found that the CO_2 tension of alveolar air, and hence, of arterial blood, is increased in sleep.

Conclusion. The acid-base balance of the blood of a normal adult at rest has been found to vary (in a regular, not random manner) during a 24-hour period due to the following factors: (a) a rise in plasma HCO_3 associated with the ingestion of food; (b) an increase in the CO_2 tension of the blood associated with sleep.

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[4] Endres, G., *Biochem. Z.*, 1923, **142**, 53.