THE RÔLE OF THE ADRENAL CORTEX IN ACUTE ANOXIA[1, 2]

By ROGER A. LEWIS,[3] GEORGE W. THORN, GEORGE F. KOEPF[4] AND SAMUEL S. DORRANCE[5]

(From the Chemical Division, Medical Clinic, The Johns Hopkins University and Hospital, Baltimore)

(Received for publication August 6, 1941)

We were led to investigate this phase of adrenal cortical function by Evans' observation (1, 2) that liver glycogen and blood glucose increased strikingly in rats exposed to low atmospheric pressure. Evans' studies clearly indicated that the phenomenon did not occur in the absence of the adrenal cortex. However, treatment with adrenal cortical hormone preparations which were then available did not restore to normal the response of adrenalectomized animals. Recently Armstrong and Heim (3) reported that hypertrophy of the adrenals occurred in rabbits which were exposed repeatedly to low atmospheric pressure. Furthermore, it has been suggested that the asthenia and hypotension which have been noted in aviators who have served continuously as pilots bear a resemblance to the clinical syndrome associated with adrenal cortical insufficiency (4).

In this investigation rats, rabbits, dogs, monkeys and human subjects have been exposed for single periods of 1.5 to 24 hours to mixtures of oxygen and nitrogen with the partial pressure of oxygen adjusted to correspond to altitudes of 11,000 to 34,000 feet (Table I). Exposure of larger animals and human subjects was accomplished at low cost by using an apparatus which permitted the mixing of nitrogen with air (5).

TABLE I

Altitude equivalent of oxygen-nitrogen mixtures used in these experiments

Oxygen concentration	Partial pressure of oxygen	Altitude above sea level
per cent of dry gas	*mm. of Hg*	*feet*
20.9	159	0
13.8	105	11,000
12.8	98	13,000
10.5	80	18,000
7.1	54	27,000
5.2	39	34,000

METHODS

Male albino rats, 150 to 200 grams in weight, of the Sprague Dawley strain, were maintained on a diet of Purina Dog Chow. Bilaterally adrenalectomized rats were treated in addition with 1 per cent sodium chloride added to the drinking water, or with one 125 mgm. pellet of desoxycorticosterone acetate[6] implanted subcutaneously, or with 1 cc. of adrenal cortical extract[7] injected daily. Rats were exposed to mixtures of oxygen and nitrogen in metabolism cages enclosed in glass bell-jars.[8] Samples of blood were obtained from the cut edge of the liver[9] at the time that specimens of liver and muscle were removed for glycogen determination. The Folin-Malmros micro method (6) was used for the blood sugar determinations. Liver and muscle glycogen were determined by the method of Good, Kramer and Somogyi (7) after the tissue had been frozen in liquid nitrogen.

Male albino rabbits, weighing approximately 2 kgm., were maintained on a diet of B–B Complete Rabbit Food Pellets (Maritime Milling Co., Inc.). Blood samples were obtained under oil from the heart. Rabbits as well as dogs and monkeys were exposed to mixtures of oxygen and nitrogen in metabolism cages enclosed in well ventilated glass chambers.[8]

Male dogs, weighing 10 to 15 kgm. were kept in metabolism cages and fed a constant diet consisting of 350 grams of raw, ground beef to which 50 cc. of Pet Milk was added. The technique used in the care of the dogs and in the collection of specimens has been described (8).

[1] An abstract of this paper was presented before the 56th Annual Meeting of the Association of American Physicians, Atlantic City, May 6, 1941.

[2] This study was aided by a grant from the Committee on Research in Endocrinology, National Research Council.

[3] John D. Archbold Fellow-in-Medicine.

[4] John H. Harris Fellow-in-Medicine.

[5] Dazian Foundation Fellow-in-Residence.

[6] The pellets of crystalline desoxycorticosterone acetate and the desoxycorticosterone acetate in sesame oil used in this study were provided through the courtesy of Dr. E. Oppenheimer of the Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

[7] The adrenal cortical extract used in this study was generously supplied by Dr. David Klein of the Wilson Co., Chicago, Illinois.

[8] The mixtures of oxygen and nitrogen were circulated through the cages at a rate which was rapid enough to prevent the accumulation of over 0.5 per cent carbon dioxide.

[9] The level of glucose in the blood from the cut edge of the liver was found to be the same as that in the blood obtained from the heart.

Bilaterally adrenalectomized dogs were maintained in good condition with 3 to 4 subcutaneously implanted pellets of desoxycorticosterone acetate (9). The methods used in the analysis of blood and urine have been described (10).

Monkeys (Macacus rhesus), weighing approximately 5 kgm., were maintained on a diet of milk, bananas, bread and raw vegetables. Samples of blood were obtained under oil from the heart.

Normal human subjects, 25 to 40 years of age, were maintained on a constant diet in which the proportions of carbohydrate, fat and protein were 5.0 : 1.5 : 1.25. The caloric content of the diet was adjusted to meet the metabolic requirements of each individual, while the mineral content was kept constant for each subject. Following a period of 5 days on the diet and an overnight fast of 15 hours, the volunteers were exposed in an oxygen tent for 5 hours. The gas mixing apparatus was modified for human use by introducing larger meters[10] and by supplying room air to the mixer under slightly positive pressure with a small air pump. The air mixture was circulated and cooled through a conventional oxygen tent. The rate of ventilation (20 to 25 liters per minute) was sufficiently rapid to keep the carbon dioxide content of the air in the tent below 1 per cent. The subjects were kept in bed before, during and for a few hours after the exposure. Samples of tent air were collected at the same time as the samples of expired air. In some instances intravenous glucose tolerance tests[11] were performed during anoxia. Blood samples for chemical analyses were removed under oil from the brachial artery. The hydrogen ion concentration of arterial blood was measured with an improved glass electrode designed for use with the Beckman pH meter.

OBSERVATIONS

Carbohydrate metabolism

1. Effect of exposure for 24 hours to oxygen at a partial pressure of 80 mm. Hg

The increase in blood sugar and liver glycogen in rats exposed to a mixture of oxygen and nitrogen of low oxygen tension (Table II) was similar to that reported by Evans in rats exposed to low barometric pressure and similar oxygen tension (1). A significant increase in the excretion of nitrogen was demonstrated only in experiments at lower oxygen tensions (discussed below). The glycogen which had accumulated in the liver of rats exposed to low oxygen tension disappeared after a subsequent 12-hour fast at approximately the same rate at which glycogen disappears from the livers of normal rats during such a fast.

TABLE II

Effect of anoxia on the carbohydrate metabolism of normal rats: exposure for 24 hours to oxygen at a partial pressure of 80 mm. Hg

	Control	Anoxia
Number of animals	4	4
Blood sugar	70*	104
mgm. per 100 cc.	(53–78)†	(87–123)
Liver glycogen	40	1,260
mgm. per 100 grams	(20–50)	(1,010–1,630)
Muscle glycogen	440	560
mgm. per 100 grams	(270–620)	(520–620)
Urine nitrogen	70	79
mgm. per 100 grams body weight per 24 hours	(pooled)	(pooled)

* The first figure indicates the average for the group.
† The figures in parentheses indicate the range for the group.

The blood sugar of dogs exposed to low oxygen tension for 24 hours did not increase. Changes in the liver glycogen of exposed dogs were not considered significant because of the large spread in the values for fasting liver glycogen of control dogs. There was, however, a definite increase in the renal excretion of nitrogen and phosphorus of dogs during exposure to low oxygen tension for 24 hours, suggesting that protein catabolism had increased somewhat. It is probable that the high liver glycogen of dogs fasted for 24 hours compared to the low liver glycogen of other species (Figure 1) is related to the marked difference in the diet of these animals (Table III), since dogs were maintained on a diet containing a high proportion of protein, whereas other experimental

TABLE III

Approximate composition of the diets which were used in preparation for exposure to low oxygen tension

Species	Per cent of dry weight		
	Carbohydrate	Protein	Fat
Rat	68	25	7
Rabbit	75	22	3
Dog	0	73	27
Monkey	80	10	10
Human	65	16	19

[10] We are indebted to Mr. M. Stockton of the American Meter Co., Albany, New York, for the meters (AL–19–1) which were used in this study.

[11] In this test 0.5 gram of glucose per kgm. of body weight was injected intravenously as a 20 per cent solution in distilled water. The flow was adjusted so that the infusion was completed in 30 minutes. Capillary blood for sugar determinations was taken in the fasting state and at 30-minute intervals during a 4-hour period following the glucose infusion.

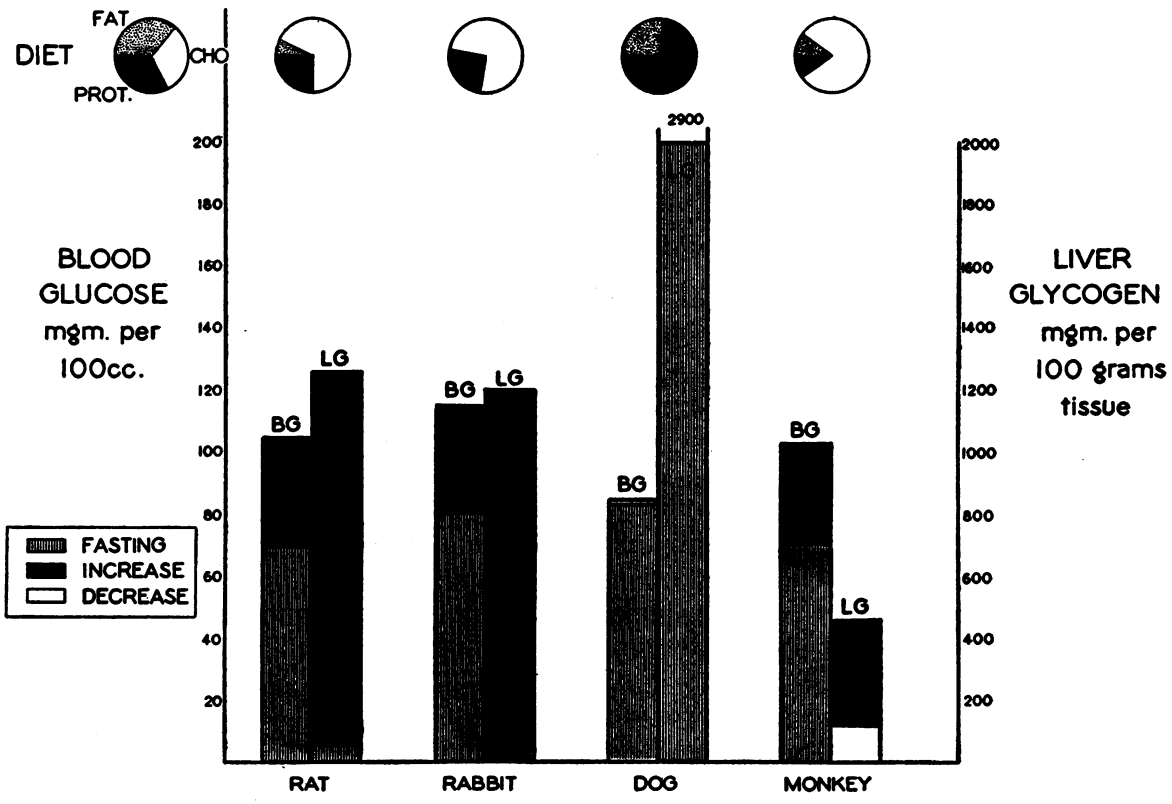

FIG. 1. THE EFFECT OF ANOXIA (24 HOURS AT 10.5 PER CENT O_2) ON LIVER GLYCOGEN AND BLOOD GLUCOSE OF VARIOUS SPECIES

The blood glucose and liver glycogen were determined in at least six animals of each species charted. The average values of animals exposed to an oxygen concentration of 10.5 per cent were charted and the average values of control animals were superimposed. The increase in blood glucose and liver glycogen is shown in solid black.

animals and human subjects were maintained on a diet rich in carbohydrate.

The blood sugar and liver glycogen of rabbits and monkeys exposed to a low oxygen tension for 24 hours were considerably greater than the blood sugar and liver glycogen of control animals that had fasted for 24 hours. Nitrogen excretion was not studied in these animals. It was not feasible to expose human subjects for 24 hours.

2. Relation of the degree of anoxia to the change in carbohydrate metabolism

When rats were exposed to various oxygen tensions for 24 hours (Figure 2) the glycogen in the liver varied inversely as the oxygen tension. Thus in 14 per cent oxygen (105 mm. Hg partial pressure) the increase in liver glycogen was slight but definite. At the other extreme of 7 per cent oxygen (54 mm. Hg partial pressure) not only liver glycogen but also blood sugar increased greatly.

3. Relation of acapnia to changes in carbohydrate metabolism during anoxia

In one series of experiments 5 per cent carbon dioxide (40 mm. Hg partial pressure) was added to the mixture of oxygen and nitrogen. In the presence of carbon dioxide during anoxia liver glycogen increased definitely but less than it did without the addition of carbon dioxide (Table IV). The fact that the addition of carbon dioxide modified the increase in carbohydrate storage is compatible with the observation that anoxemia is decreased by the administration of carbon dioxide (11).

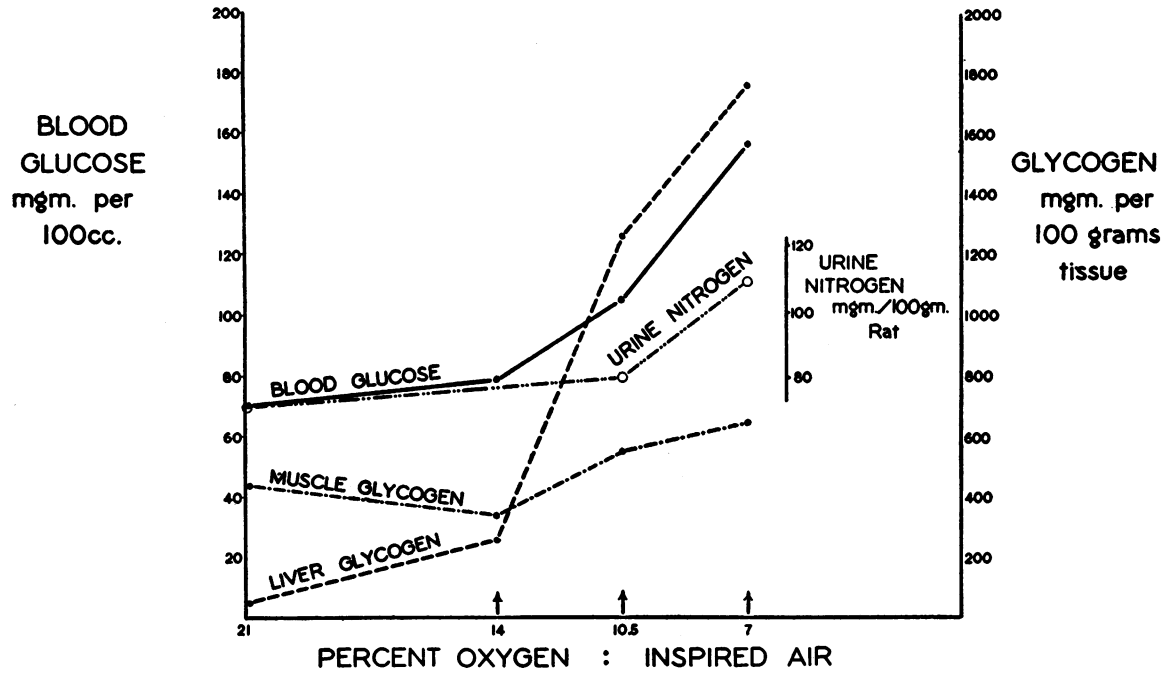

FIG. 2. THE EFFECT OF ANOXIA (24 HOURS) ON BLOOD GLUCOSE, LIVER AND MUSCLE GLYCOGEN OF NORMAL RATS

TABLE IV

Changes in carbohydrate metabolism during anoxia: the effect of adding carbon dioxide to the mixture of oxygen and nitrogen

Number of rats used	Carbon-dioxide partial pressure	Oxygen partial pressure	Blood sugar	Liver glycogen	Muscle glycogen
	mm. Hg	*mm. Hg*	*mgm. per 100 cc.*	*mgm. per 100 grams*	*mgm. per 100 grams*
4	0	159	70	60	440
4	0	80	104	1,260	560
4	40	80	91	500	480

TABLE V

Effect of anoxia on the carbohydrate metabolism of adrenalectomized rats: exposure for 24 hours to oxygen at a partial pressure of 80 mm. Hg*

	Control (adrenalectomized)	Anoxia (adrenalectomized)
Number of animals	3	3
Blood sugar	54†	42
mgm. per 100 cc.	(45–66)**	(36–50)
Liver glycogen	30	20
mgm. per 100 grams	(20–40)	(20–30)
Muscle glycogen	250	90
mgm. per 100 grams	(120–380)	(70–100)
Urine nitrogen	74	55
mgm. per 100 grams body weight per 24 hours	(pooled)	(pooled)

* Adrenalectomized rats were maintained with 1 per cent sodium chloride added to the drinking water.

† The first figure indicates the average for the group.

** The figures in parentheses indicate the range for the group.

4. Relation of the adrenal cortex to carbohydrate metabolism during anoxia

In adrenalectomized rats exposed to low oxygen tension (80 mm. Hg partial pressure) for 24 hours liver glycogen did not increase (Table V). This confirms the studies of Evans (1, 2). Muscle glycogen and blood glucose of exposed adrenalectomized rats were below the low fasting level of unexposed adrenalectomized animals. A number of adrenalectomized rats maintained on sodium chloride did not survive the exposure.

5. Effect of adrenal cortical hormone therapy on the carbohydrate metabolism of adrenalectomized rats during anoxia

Injections of aqueous adrenal cortical extract at hourly intervals or a suspension of this material in oil at 6-hour intervals were followed by a rise in the blood sugar and liver glycogen of adrenalectomized rats during anoxia (Figures 3, 4). With this treatment adrenalectomized rats were enabled to tolerate a 24-hour exposure to an oxygen partial pressure of 80 mm. Hg without signs of hypoglycemia. Treatment with adequate quantities of potent *adrenal cortical extract thus restored the ability of adrenalectomized animals to respond to anoxia in a manner comparable to con-*

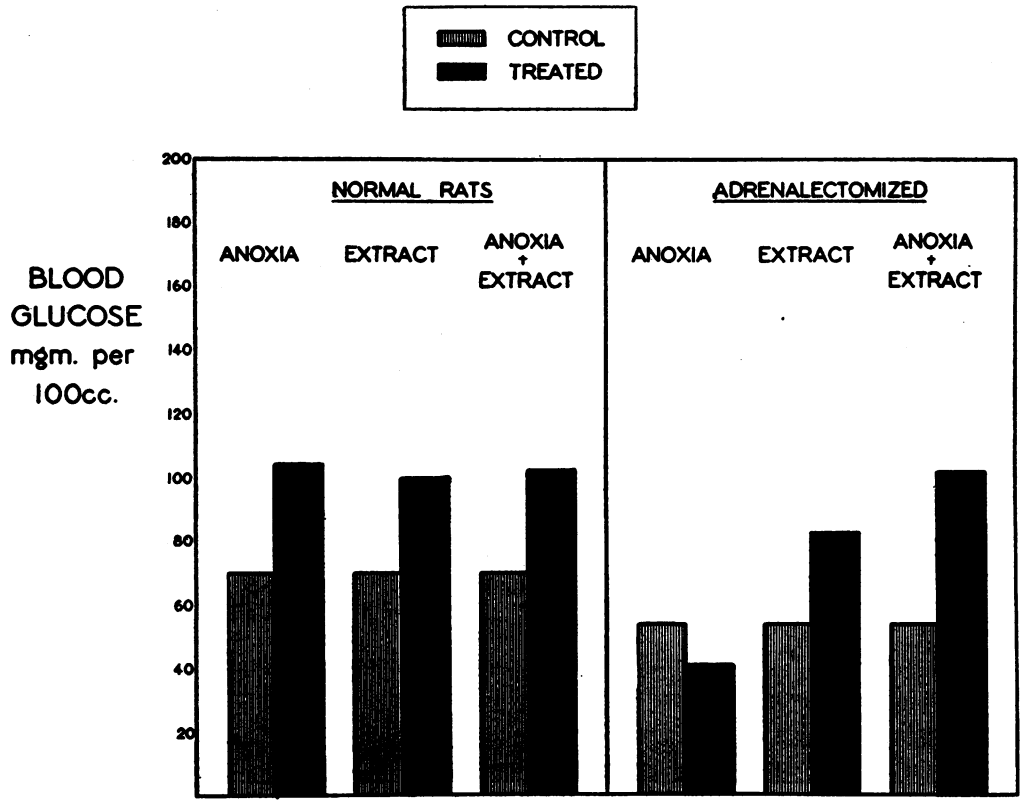

FIG. 3. EFFECT OF ANOXIA (24 HOURS AT 10.5 PER CENT O_2) AND ADRENAL CORTICAL EXTRACT TREATMENT ON BLOOD GLUCOSE

trols. There was no increase in the blood sugar or liver glycogen of adrenalectomized rats treated with sodium chloride or desoxycorticosterone acetate and exposed to low oxygen tension.

6. Effect of adrenal cortical hormone therapy on the carbohydrate metabolism of normal unexposed rats

It has been shown by Long *et al.* (12) that the administration of certain adrenal cortical hormones tends to augment carbohydrate storage and nitrogen excretion. The liver glycogen content is of the same order of magnitude in normal and adrenalectomized rats treated with adrenal cortical extract or 11-dehydro-17-hydroxycorticosterone [12] as it is in normal rats exposed to low oxygen tensions (Table VI). The increase in nitrogen excretion of normal and adrenalectomized animals treated with 11-dehydro-17-hydroxycorticosterone exceeded the increase observed during anoxia.

TABLE VI

The effect of adrenal cortical hormone therapy on carbohydrate metabolism of rats

Treatment	Control	Anoxia, 24 hours at 80 mm. Hg oxygen partial pressure	Adrenal cortical extract, 10 cc. in 1 cc. oil	11-dehydro-17-hydroxy-corticosterone, 6 mgm. in 1 cc. oil	Desoxy-corticosterone acetate, 6 mgm. in 1 cc. oil
Number of animals	3	3	3	1	3
Blood sugar *mgm. per 100 cc.*	70	104	108	105	63
Liver glycogen *mgm. per 100 cc.*	60	1,260	1,870	1,240	20
Musle glycogen *mgm. per 100 cc.*	440	560	300	270	140
Urine nitrogen *mgm. per 100 grams body weight per 24 hours*	70	79	78	103	68

7. Effect of exposure for 1.5 to 6 hours to reduced oxygen tension

Preliminary observations suggested that in some animals a hypoglycemic phase might precede the

[12] We are indebted to Dr. E. C. Kendall of the Mayo Clinic, Rochester, Minnesota, for the crystalline 11-dehydro-17-hydroxycorticosterone (Compound E).

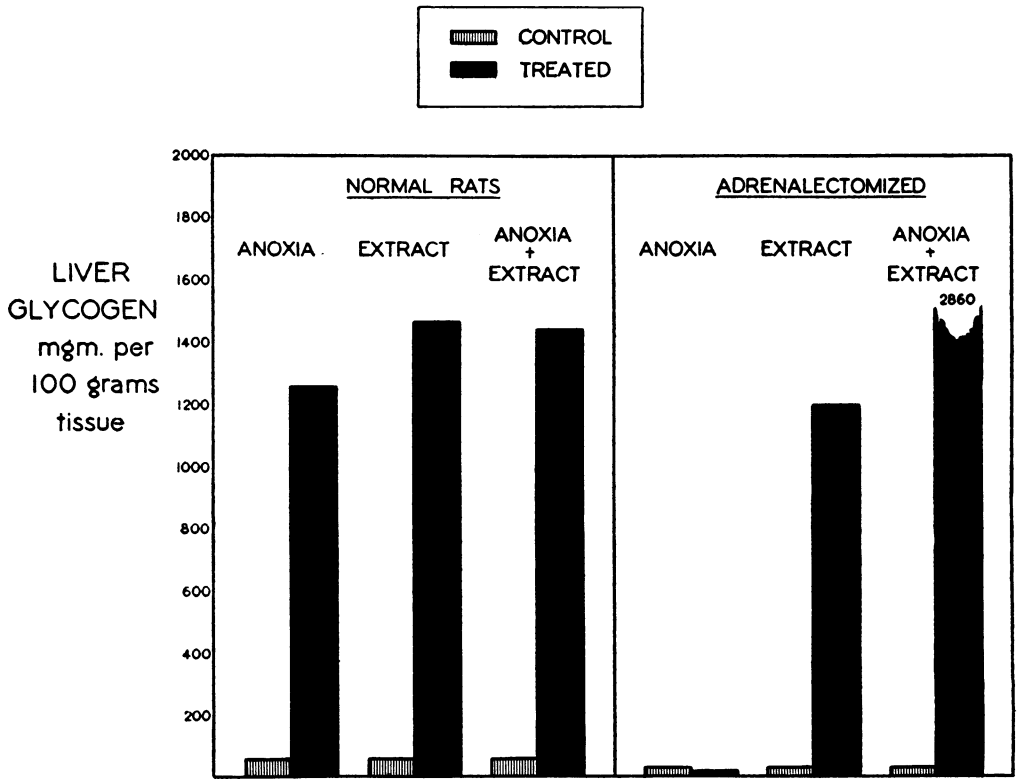

FIG. 4. EFFECT OF ANOXIA (24 HOURS AT 10.5 PER CENT O_2) AND ADRENAL CORTICAL EXTRACT TREATMENT ON LIVER GLYCOGEN

increase in carbohydrate stores that occurs after 24 hours of anoxia. A detailed study was made of the blood sugar, liver and muscle glycogen of rats exposed for 1.5, 3, 6, and 12 hours to reduced oxygen tension (59 mm. Hg partial pressure). In the early hours of exposure no significant change in blood sugar was observed. However, the *liver glycogen was considerably decreased during the early phase of exposure* when compared to the liver glycogen of normal rats fasted for a similar period (Figure 5).

An exposure period of 1.5 hours was selected for the study of the effect of extremely low oxygen tension on the blood sugar and liver glycogen of normal rats. The blood glucose during anoxia did not fall until the partial pressure of oxygen was reduced to 39 mm. Hg which was sufficient to reduce liver glycogen to a minimum (Figure 6). At this greatly reduced oxygen tension, fatal convulsions frequently occurred. However, very low liver glycogen was noted in animals that did not have convulsions.

8. Effect of anoxia (5 hours) on normal human subjects

Eight normal male subjects were exposed to an average oxygen tension of 98 mm. Hg after a 15-hour fast. Metabolic studies made during anoxia were compared to similar studies made during a comparable control period. All of the subjects exhibited most of the signs and symptoms which have been described in subjects exposed to low barometric pressure (Table VII). Severe frontal headache was noted in all but one case. Vasomotor collapse occurred in two subjects.

The significant metabolic changes are shown in Table VIII. Cyanosis was apparent in all cases, although the average arterial oxygen saturation was 83 per cent. Pulmonary ventilation was increased approximately 25 per cent. A definite decrease in hydrogen ion concentration was noted. Changes in oxygen consumption varied in different individuals but the average oxygen consumption of the group was increased about 5 per cent. Despite the fact that there was no decrease in the

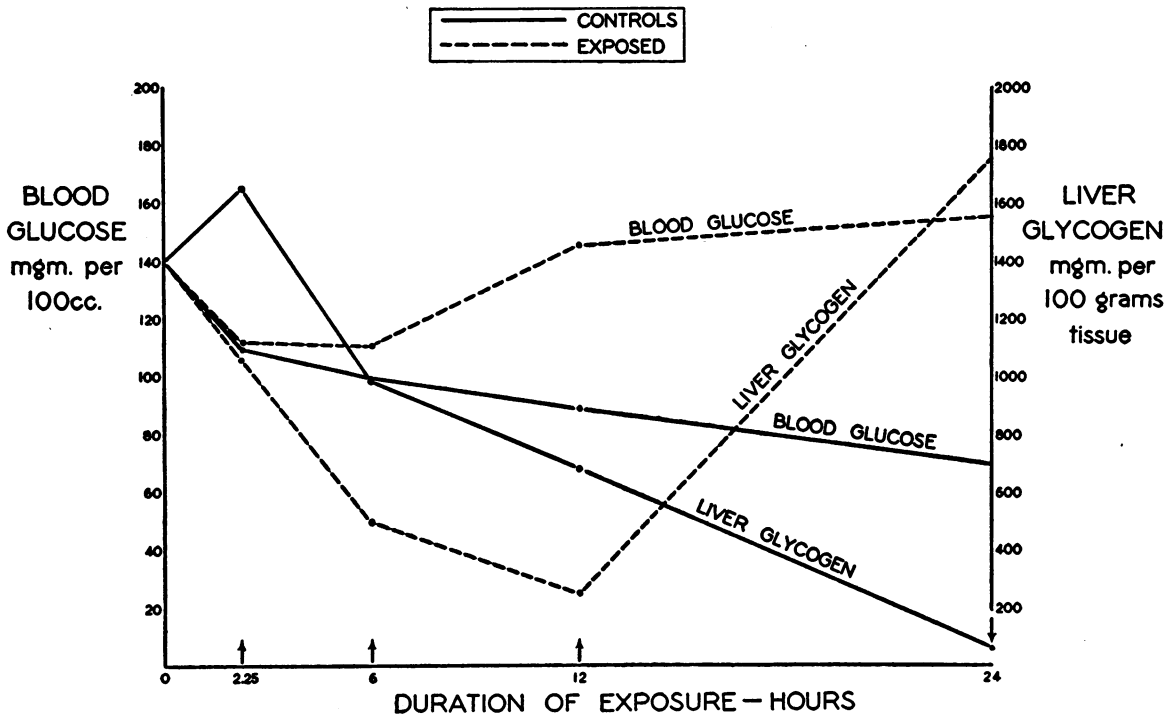

FIG. 5. THE EFFECT OF ANOXIA (7 PER CENT O_2) ON BLOOD GLUCOSE AND LIVER GLYCOGEN OF NORMAL RATS

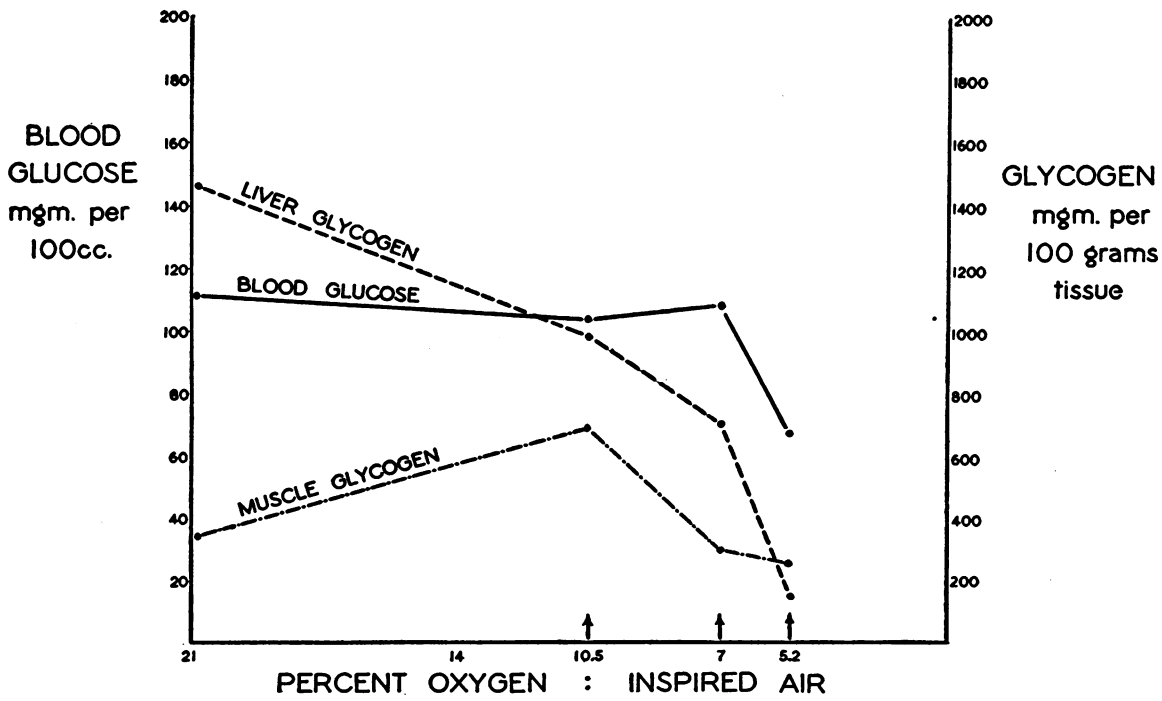

FIG. 6. THE EFFECT OF ANOXIA (1½ HOURS) ON BLOOD GLUCOSE, LIVER AND MUSCLE GLYCOGEN OF NORMAL RATS

TABLE VII

Effect on normal subjects of exposure to oxygen at a partial pressure of 98 mm. Hg for 5 hours

Subject	H.A.	S.D.	C.G.	R.G.	B.K.	G.K.	R.L.	G.T.	Summary
Oxygen partial pressure in *mm. Hg*	91	86	101	97	102	93	101	98	
Carbon dioxide partial pressure in *mm. Hg*	5	1	5	4	4	4	7	5	
Hyperventilation	++++	+++	+++	++	++	+++	++	++	8
Tachycardia	++	++	+	++++	++	++	+	+	8
Cyanosis	++	+++	++	++	+++	++	++	++	8
Frontal headache	+	+++	0	+++	++	+	++	++++	7
Mental exhilaration	+	0	+	++	0	+++	++	+++	6
General weakness	0	+++	0	0	++	0	0	+	3
Fall in blood pressure	0	0	0	0	++	0	0	++++	2
Syncope	0	0	0	0	+++	0	0	++++	2
Precordial discomfort	0	++	0	0	0	0	0	++	2
Nausea or vomiting	0	0	+	0	0	0	0	0	1

TABLE VIII

Metabolic changes in normal subjects exposed to oxygen at a partial pressure of 98 mm. Hg for 5 hours

	Control (8)	Anoxia (8)
Arterial oxygen saturation, *per cent.*		83
Pulmonary ventilation, *liters per hour*	304	377
Respiratory rate, *respirations per minute*		16
Oxygen consumption, *cc. per minute*	248	258
Respiratory quotient	78	81
Arterial pH	7.49	7.58
Serum chloride, *m. eq. per liter*	102.7	104.5
Serum sodium, *m. eq. per liter*	138.4	138.0
Serum potassium, *m. eq. per liter*	5.6	5.4
Serum inorganic phosphorus, *mgm. per 100 cc.*	3.9	2.9
Serum nonprotein nitrogen, *mgm. per 100 cc.*	27	24
Blood sugar, *mgm. per 100 cc.*	90	91
Urinary excretion of chloride, *m. eq. per hour*	6.5	5.9
Urinary excretion of sodium, *m. eq. per hour*	6.5	6.6
Urinary excretion of potassium, *m. eq. per hour*	3.8	3.7
Urinary excretion of phosphorus, *grams per hour*	0.023	0.012
Urinary excretion of nitrogen, *grams per hour*	0.600	0.518
Urinary excretion of water, *cc. per hour*	142	137

average oxygen consumption, there was a definite and consistent decrease in nitrogen excretion, without any increase in the serum nonprotein nitrogen. No significant change was noted in the fasting blood sugar, or in the curve following intravenous administration of glucose. Slight changes in the "T" waves of the electrocardiogram taken during anoxia were not prevented by the administration of glucose.

Mineral metabolism

1. Effect of exposure for 24 hours to low oxygen tension

In rabbits and monkeys the electrolyte concentrations in the blood of a control group of animals were compared with the concentrations in a group of animals exposed to low oxygen tensions for 24 hours. With dogs it was possible to obtain adequate blood samples from the same animal prior to and following anoxia and at the same time to determine the renal excretion of electrolytes.

A decrease in serum bicarbonate (2 to 5 m.eq per liter) and an increase in serum chloride (4 to 8 m.eq. per liter) were noted following exposure to low oxygen tension for 24 hours. No significant change in the serum sodium or serum potassium was observed. When the exposure was associated with vomiting there was a rise in the nonprotein nitrogen. *During the 24-hour exposure the renal excretion of potassium, sodium and chloride of normal dogs was greatly increased* (Figure 7). A less marked increase in the excretion of inorganic phosphorus and total nitrogen was also noted. Similar but less exact changes were observed in the urine of normal rats exposed to low oxygen tensions for 24 hours.

2. Relation of the adrenal cortex to changes in mineral metabolism during exposure to low oxygen tension (80 mm. Hg) for 24 hours

It was possible to study the effect of anoxia upon two adrenalectomized dogs maintained with

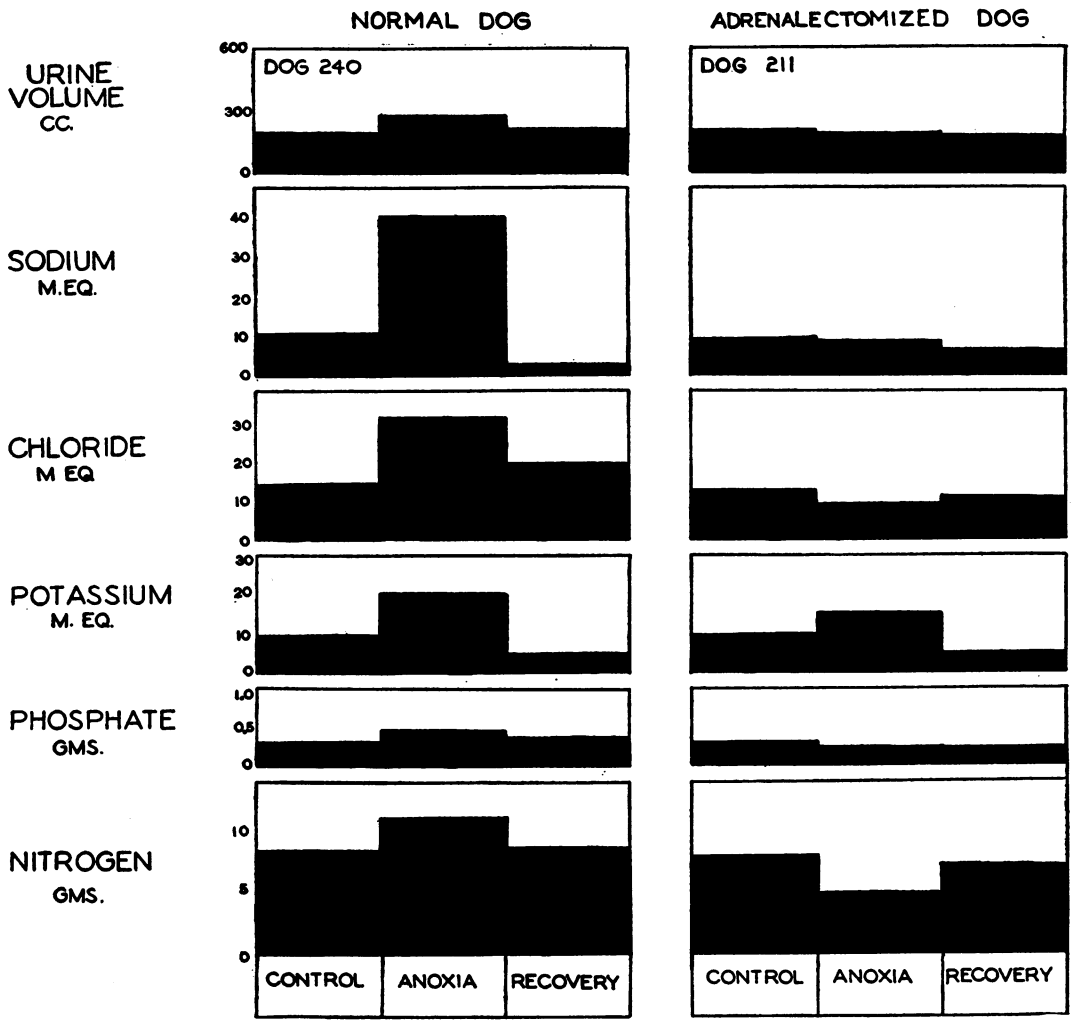

FIG. 7. THE RENAL EXCRETION OF NITROGEN AND ELECTROLYTES OF NORMAL AND ADRENALECTOMIZED DOGS BEFORE, DURING, AND AFTER EXPOSURE TO 10.5 PER CENT OXYGEN FOR 24 HOURS

desoxycorticosterone acetate and upon adrenalectomized rats maintained with sodium chloride or desoxycorticosterone. The changes in blood chemistry which occurred in the adrenalectomized dogs exposed to low oxygen tension were similar to the changes in blood chemistry that occurred in normal dogs during anoxia. There was also a striking increase in the renal excretion of potassium in adrenalectomized animals that resembled the effect of anoxia upon the excretion of potassium in normal animals. The slight increase in nitrogen and phosphorus excretion and the marked increase in sodium and chloride excretion that were noted in normal dogs failed to occur in adrenalectomized animals during exposure to anoxia. The failure of adrenalectomized animals to excrete an increased quantity of nitrogen and phosphorus during anoxia is consistent with the observation that, in the absence of the adrenal cortex, protein catabolism does not increase during anoxia. The reason for the absence of any increase in the excretion of sodium and chloride, despite the large increase in the excretion of potassium, was not obvious. The failure of adrenalectomized animals to excrete excessive amounts of sodium and chloride during anoxia did not appear to be due to the maintenance dose of desoxycorticosterone acetate since adrenalectomized rats maintained on sodium chloride reacted in the same manner and since normal animals treated with desoxycorticosterone ace-

tate excreted large amounts of sodium and chloride during anoxia. The absence of any rise in the blood nonprotein nitrogen or serum potassium indicates that neither renal failure nor adrenal insufficiency was responsible for the failure of adrenalectomized animals to excrete increased quantities of sodium and chloride during anoxia.

3. Effect of the "carbohydrate-regulating" factors of the adrenal cortex on the renal excretion of electrolytes at atmospheric oxygen tension

From other studies (13) it was suspected that the increased secretion of adrenal cortical substances possessing "carbohydrate-regulating" activity might increase the excretion of sodium and chloride. The effect of treatment with 11-dehydro-17-hydroxycorticosterone on the renal excretion of normal and adrenalectomized animals was therefore studied (Figure 8). In the normal dog there was an increased excretion of nitrogen and phosphorus, and a more strikingly increased excretion of sodium, chloride and water. *The marked increase in the excretion of potassium which occurred in normal and adrenalectomized animals during anoxia did not occur during treatment with 11-dehydro-17-hydroxycorticosterone.* It was of interest to note that an adrenalectomized

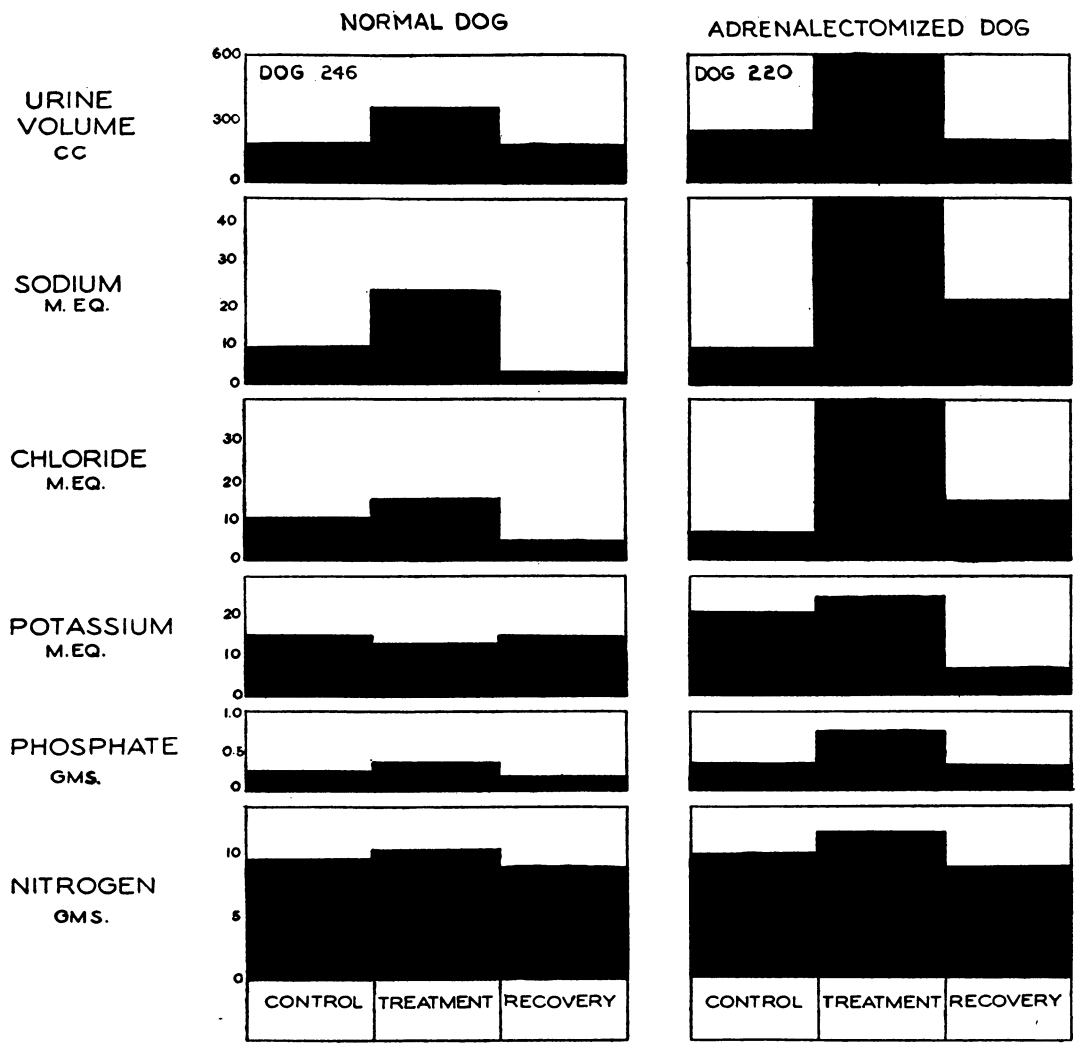

FIG. 8. THE RENAL EXCRETION OF NITROGEN AND ELECTROLYTES OF NORMAL AND ADRENALECTOMIZED DOGS BEFORE, DURING, AND AFTER TREATMENT WITH 11-DEHYDRO-17-HYDROXYCORTICOSTERONE

dog treated with 25 mgm. of 11-dehydro-17-hydroxycorticosterone showed the same changes in sodium, chloride, phosphorus and nitrogen excretion that were observed in normal animals during anoxia. The serum concentration of sodium, potassium, chloride and carbon dioxide was not affected by treatment with 11-dehydro-17-hydroxycorticosterone, although a rise in nonprotein nitrogen occurred.

4. Effect of anoxia (5 hours) on the mineral metabolism of human subjects

Changes in the serum concentration and renal excretion of electrolytes were studied in eight normal subjects exposed to an average oxygen tension of 98 mm. Hg (Table VIII). There was a distinct reduction in hydrogen ion concentration. There was no change in the renal excretion of sodium and potassium, although the nonprotein nitrogen and phosphorus excretion were slightly reduced.

In contrast to the increase in nitrogen and phosphorus excretion which was observed during the 24-hour period of anoxia in animal experiments, the excretion of these substances by human subjects during a 5-hour period of anoxia was decreased.

DISCUSSION

The observations which compose this report were made by exposing experimental animals and human subjects to an atmosphere of reduced oxygen concentration at normal atmospheric pressure. No effort was made to simulate the changes in total pressure which occur at high altitudes. It is known, however, that the same degree of arterial oxygen unsaturation occurs at moderately reduced oxygen tension whether this is due to low oxygen concentration or low total pressure (14). At greatly reduced pressures a difference in gaseous exchange, due to the increase in the mean free path of the gas molecules, may occur (15).

The difference between liver glycogen of rats exposed to low oxygen tension and liver glycogen of control rats was larger than the difference noted in other species. This is due to the fact that, whereas the liver glycogen of rats fasted for 24 hours is uniformly almost negligible, in other species it is impossible to effect a total depletion of liver glycogen by starvation for 24 hours.

When dogs were exposed to low oxygen tension or treated with adrenal cortical extract neither blood glucose nor liver glycogen rose significantly. This species peculiarity may be due to the fact that dogs were fed a diet composed almost exclusively of protein and fat. Thus, whether dogs were fed, fasted, exposed to low oxygen tension, or treated with adrenal cortical extract, the same type of foodstuff was being utilized. A similar phenomenon has been described in rats maintained on a diet rich in protein (16).

A delay in the appearance of increased nitrogen excretion which occurs during anoxia was described by Brunquist *et al.* (17). This observation is consistent with the fact that protein catabolism is diminished during the early phase of anoxia and increased only in the later phase of anoxia. Furthermore, in the absence of the adrenal cortex, no increase in protein catabolism occurs and the nitrogen excretion during the entire 24-hour period of anoxia is less than during a similar control period.

The depletion of liver glycogen that occurs during the initial phase of anoxia was described by Paul Bert in 1878: "quand la depression est forte et qu'elle agit pendant longtemps (5 to 6 hours) le sucre diminue plus ou moins dans le foie" (18). During exposures to moderately reduced oxygen tension blood glucose is maintained at a normal level at the expense of liver glycogen. During exposures to greatly reduced oxygen tension a fall in blood glucose occurs after the liver glycogen has been exhausted. These experiments indicate that the primary effect of anoxia is exactly the opposite of the effect of adrenal cortical "carbohydrate-regulating" hormone, although the effect upon liver glycogen of a 24-hour exposure to low oxygen tension is similar to the effect of treatment with this hormone. In adrenalectomized animals the primary effect of anoxia continues throughout the exposure and the animals frequently succumb with hypoglycemic convulsions. In normal animals increased utilization of carbohydrate may continue throughout the period of exposure, but when glycogen is exhausted protein sources come to the rescue and in the end there is an accumulation of glycogen stores.

Although most of the studies here reported were made at oxygen tensions which correspond to 18,000 feet, it is interesting to note that definite

changes in carbohydrate metabolism were noted at oxygen tensions that correspond to 11,000 feet. It was not feasible to expose human subjects to anoxia for 24 hours. However, the blood glucose of monkeys increased during a 24-hour exposure to anoxia. This elevation of blood glucose is similar to that which occurs in patients suffering from the anoxemia of carbon monoxide poisoning (19). Depletion of liver glycogen probably accounts for the increased susceptibility of animals to insulin during the early phase of exposure to low oxygen tension (20).

The reactions of human subjects to low oxygen concentration were similar to the reactions which occur during exposure to low barometric pressure. Two subjects had a momentary lapse of consciousness toward the end of the 5-hour exposure, one after an unsuccessful arterial puncture. The syncope, characterized by a slowing of the pulse and a fall in blood pressure, was followed by a rise in blood glucose and serum nonprotein nitrogen. However, both of the individuals who experienced these attacks had also experienced similar reactions while on automobile or boat trips. Although both of the subjects who fainted were in the group of four that were not given intravenous glucose, it was not thought that this was of particular signifiance because the administration of glucose was not associated with improvement in subjective sensations. Electrocardiographic changes during anoxia were not restored to normal by the administration of glucose, although changes in the electrocardiogram during anoxia have been correlated with abnormalities of carbohydrate metabolism (21). The reduction in the hydrogen ion concentration of the arterial blood may have been due to the fact that human subjects were kept in bed under basal conditions throughout the exposure periods.

Changes in oxygen consumption were too small to be of significance. On the other hand, despite the absence of a fall in the oxygen consumption, a consistent fall in nitrogen excretion was observed. No increase in the serum nonprotein nitrogen occurred, suggesting that during the early phase of anoxia in human subjects there is a shift of metabolism from protein to carbohydrate similar to that demonstrated in experimental animals.

There are few data available relative to the effect of low oxygen tension upon the excretion of electrolytes with the exception of Sundstroem's observations (22) of subjects on mountain expeditions. A consistent increase in potassium excretion was found in all animals, normal or adrenalectomized, that were exposed to low oxygen tension for 24 hours. Part of the increased excretion of potassium during exposure to low oxygen tension may be associated with the rapid loss of glycogen from the liver which occurs initially in normal and adrenalectomized animals. It appears that the increased renal excretion of potassium which occurs during exposure to anoxia represents for the most part a direct effect of anoxemia and not an effect mediated by the adrenal cortex inasmuch as it occurs in both normal and adrenalectomized animals exposed to anoxia and since little or no increase in potassium excretion followed the administration of adrenal cortical "carbohydrate-regulating" factor.

The fact that normal animals exposed to low oxygen tension for 24 hours excreted large quantities of sodium and chloride, whereas adrenalectomized animals similarly exposed did not excrete an increased quantity of sodium and chloride, was thought at first to be due to differences in the respiratory response of these animals. Cope has demonstrated such a difference (23) and Hoffman *et al.* (24) have reported an unusual encephalo-electrical sensitivity to hyperventilation in the majority of a group of twenty-five patients with Addison's disease. However, the magnitude of the shift in serum bicarbonate and chloride ions during anoxia was of the same order of magnitude in normal and adrenalectomized animals.

From the observations of Ingle and Thorn (13) who compared the effect of desoxycorticosterone acetate and 11-dehydro-17-hydroxycorticosterone in partially depancreatized rats, it was suspected that an increase in sodium and chloride excretion might be produced by adrenal cortical "carbohydrate-regulating" hormone. Recent studies (25) confirm the fact that treatment with certain adrenal cortical steroid compounds which possess a hydroxyl group on C_{17} induces sodium and chloride excretion in contrast to the well-known "sodium-retaining" effect of such adrenal cortical steroid compounds as corticosterone and 11-desoxycorticosterone. In contrast to the retention of water that occurs during treatment with "so-

dium-retaining" sterols, there was a considerable increase in the excretion of water during treatment with "carbohydrate-regulating" factor. The increase in sodium and chloride excretion that was observed was greater than the increase in water excretion, but the concentration of sodium and chloride excreted bears a close relation to the concentration of these substances in the body fluids. The excretion of chloride is small in proportion to that of sodium, possibly because chloride is retained to replace carbon dioxide lost through overventilation.

It appears consistent that during anoxia adrenalectomized animals which are unable to increase protein catabolism by increasing the secretion of adrenal cortical "carbohydrate-regulating" factor do not exhibit the increase in sodium and chloride excretion that this hormone induces.

There is no evidence that the effect of adrenal cortical "carbohydrate-regulating" factor in increasing the excretion of sodium and chloride during anoxia is beneficial, whereas its effect in increasing protein catabolism might be a favorable reaction since during exposure to low oxygen tension there is an increased utilization of carbohydrate. Furthermore, the work of McFarland and Forbes (26) suggests that an increase in carbohydrate may alleviate to some extent the deleterious effects of anoxemia upon higher functions.

SUMMARY

1. Normal human subjects exposed to an average oxygen tension of 98 mm. Hg for 5 hours had an average arterial oxygen saturation of 83 per cent. Despite the fact that there was no decrease in the oxygen consumption there was a definite and consistent decrease in the nitrogen excretion.

2. Experimental animals exposed to low oxygen tensions for similar periods showed a depletion of liver glycogen that was not associated with a fall in blood glucose level unless the anoxia was very severe.

3. Exposure of normal animals to low oxygen tension for 24 hours was associated with a rise in blood glucose and liver glycogen (except in dogs), and an increase in the renal excretion of nitrogen (in all animals).

4. Adrenalectomized animals succumbed when exposed to oxygen tensions that normal animals were able to withstand, and no increase in blood glucose, liver glycogen or nitrogen excretion occurred.

5. Treatment with "carbohydrate-regulating" factor of the adrenal cortex enabled adrenalectomized animals to withstand exposure to an otherwise fatal oxygen tension, and resulted in an increase in blood glucose, liver glycogen and nitrogen excretion.

6. Normal animals exposed to low oxygen tensions for 24 hours showed a slight increase in nitrogen and phosphorus excretion and a marked increase in sodium, chloride and potassium excretion.

7. Adrenalectomized animals exposed to low oxygen tensions for 24 hours exhibited a striking increase in potassium excretion but no increase in nitrogen, phosphorus, sodium or chloride excretion.

8. Treatment of normal and adrenalectomized animals with "carbohydrate-regulating" factor of the adrenal cortex was followed by a striking increase in sodium, chloride and water excretion but no significant increase in potassium excretion.

CONCLUSION

During the initial phase of anoxia there appears to be an increased utilization of carbohydrate. A normal blood glucose level is maintained at the expense of liver glycogen stores. Successful adaptation to continued exposure to low oxygen tension depends in part upon an increase in protein catabolism with a subsequent rise in carbohydrate stores and increase in nitrogen excretion. These changes do not occur in the absence of the adrenal cortex. Acute anoxia is accompanied by a rise in the chloride ion concentration of the serum and by a fall in the hydrogen ion concentration (rise in pH) of the blood. Prolonged anoxia (24 hours) leads to a marked increase in the renal excretion of sodium, chloride and potassium. The increase in potassium excretion appears to be accounted for principally by factors other than the adrenal cortex, whereas the increase in sodium and chloride excretion appears to be mediated by the "carbohydrate-regulating" factor of the adrenal cortex.

The authors wish to express their appreciation to Doctor E. Nichols for taking and reading electrocardiograms, to Miss M. Caldwell and Mrs. F. Hesser for assistance in the metabolic studies on human subjects,

and to Doctors R. L. Grief, B. Katzin, H. Aranow and C. L. Gemmil who kindly volunteered as subjects for exposure to low oxygen tension.

BIBLIOGRAPHY

1. Evans, G., The effect of low atmospheric pressure on the glycogen content of the rat. Am. J. Physiol., 1934, **110**, 273.
2. Evans, G., The adrenal cortex and endogenous carbohydrate formation. Am. J. Physiol., 1936, **114**, 297.
3. Armstrong, H. G., and Heim, J. W., Effect of repeated daily exposures to anoxemia. J. Aviation Med., 1938, **9**, 92.
4. Armstrong, H. G., Principles and Practice of Aviation Medicine. Williams and Wilkins Co., Baltimore, 1939, pp. 286–288.
5. Lewis, R. A., and Koepf, G. F., An apparatus for producing constant gas mixtures. Science, 1941, **93**, 407.
6. Folin, O., and Malmros, H., An improved form of Folin's micro method for blood sugar determinations. J. Biol. Chem., 1929, **83**, 115.
7. Good, C. A., Kramer, H., and Somogyi, M., The determination of glycogen. J. Biol. Chem., 1933, **100**, 485.
8. Thorn, G. W., Engel, L. L., and Eisenberg, H., The effect of corticosterone and related compounds on the renal excretion of electrolytes. J. Exper. Med., 1938, **68**, 161.
9. Thorn, G. W., Engel, L. L., and Eisenberg, H., Treatment of adrenal insufficiency by means of subcutaneous implants of desoxycorticosterone acetate. Bull. Johns Hopkins Hosp., 1939, **64**, 155.
10. Thorn, G. W., Howard, R. P., and Emerson, K., Jr., Treatment of Addison's disease with desoxycorticosterone acetate. J. Clin. Invest., 1939, **18**, 449.
11. McFarland, R. A., and Dill, D. B., A comparative study of the effects of reduced oxygen pressure on man during acclimatization. J. Aviation Med., 1938, **9**, 18.
12. Long, C. N. H., Katzin, B., and Fry, E. G., The adrenal cortex and carbohydrate metabolism. Endocrinology, 1940, **26**, 309.
13. Ingle, D. J., and Thorn, G. W., A comparison of the effects of 11-desoxycorticosterone acetate and 17-hydroxy-11-dehydrocorticosterone in partially depancreatized rats. Am. J. Physiol., 1941, **132**, 670.
14. Dill, D. B., Edwards, H. T., and Robinson, S., Pulmonary gaseous exchanges at low barometric pressure and in air mixed with nitrogen. J. Aviation Med., 1939, **10**, 3.
15. Haldane, J. S., and Priestley, J. G., Respiration. Second Edition. Yale University Press, New Haven, 1935, p. 301.
16. Mirski, A., Rosenbaum, I., Stein, L., and Wertheimer, E., On the behaviour of glycogen after diets rich in protein and in carbohydrate. J. Physiol., 1938, **92**, 48.
17. Brunquist, E. H., Schneller, E. J., and Loevenhart, A. S., The effects of anoxemia on nitrogen metabolism. J. Biol. Chem., 1924, **62**, 93.
18. Bert, P., La Pression Barometrique. G. Masson, Paris, 1878.
19. Peters, J. P., and Van Slyke, D. D., Quantitative Clinical Chemistry, Volume I, Interpretations. Williams and Wilkins Co., Baltimore, 1931, p. 191.
20. Glickman, N., and Gellhorn, E., The effect of oxygen deficiency on the sensitivity of rats to insulin. Am. J. Physiol., 1938, **121**, 358.
21. Larsen, K., Effect of anoxemia on the human electrocardiogram. Acta. med. Scandinav., supp. 1936, **78**, 141.
22. Sundstroem, E. S., Studies on adaptation of man to high altitudes, V. Univ. California Publications Physiol., 1919, **5**, 121.
23. Cope, O., Personal communication.
24. Hoffman, W. C., Lewis, R. A., and Thorn, G. W., The electroencephalogram in Addison's disease. (To be published.)
25. Thorn, G. W., Engel, L. L., and Lewis, R. A., The effect of 17-hydroxycorticosterone and related adrenal cortical steroids on sodium and chloride excretion. Science, 1941, **94**, 348.
26. McFarland, R. A., and Forbes, W. H., The effects of variations in the concentration of oxygen and of glucose on dark adaptation. J. General Physiol., 1940, **24**, 69.