PC & UC Levels in Mice Exposed toAcute Cold

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ABSTRACT

by

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PLASMA AND URINARY CORTICOSTEROIDS LEVELS IN MICE EXPOSED TO ACUTE COLD (-10°C)

The plasma and urinary corticosteroids, haemoglobin, blood urea nitrogen and plasma creatinine were determined in CF_1 female mice before, during and after exposure to -10°C.

Plasma corticosterone levels increased significantly soon after exposure, remained significantly elevated even in comatose animals and for an hour during recovery in the warmth, and returned to normal levels a day after the stress. The pattern of change was similar in mice exposed twice, though the deviations from the control levels were less. Urinary corticosteroids were depressed the first day following each exposure, and more so following the second one, with the values tending to return to normal by the seventh day. Haemoglobin levels were not reliably changed during the first exposure, but were elevated during the second. Blood urea nitrogen and plasma creatinine levels were elevated after each exposure, and the latter remained elevated for at least 7 days following the second cold exposure.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ACE	Adrenocortical extracts
ACTH	Adrenocorticotropic hormone
Adrex	Adrenalectomy
adrexed	Adrenalectomized
Aldo	Aldosterone
ASD	Delta 4-androstenedione
B	Corticosterone
BMR	Basal metabolic rate
BUN	Blood urea nitrogen
BW	Body weight
CBG	Corticosteroid-binding globulin
CC	Circulating corticosterone
CCS	Circulating corticosteroid (corticoid)
CHO	Carbohydrate
Cl	Chloride
CO_2	Carbondioxide
CPB	Competitive protein binding
CRF	Corticotrophin releasing factor
CS	Corticosteroid
CT	Colonic temperature
DHE	Dehydroepiandrosterone
DOC	11-desoxycorticosterone
E	Exposure
F	Cortisol (hydrocortisone)
GCC	Glucocorticoid
Hb	Haemoglobin
H_2O	Water
HCO_3	Bicarbonate
hypox	Hypophysectomy
hypoxed	Hypophysectomized
I	Iodine
I^{131}	Radio active iodine
K	Potassium
mcg	Micro gram
mgs	Milligrams
ml	Milliliter
MR	Metabolic rate
mμc	Milli micro curie
Na	Sodium
ng	Nanogram
O_2	Oxygen
P	Phosphorus
P^{32}	Radio phosphorus

Continued .....

PB	Protein binding
PC	Plasma corticosterone
PRA	Plasma renin activity
RAS	Renin-angiotensin system
S	11-deoxycortisol
SD	Standard deviation
T	Time
TSH	Thyroid-stimulating hormone
μc	Microcurie
UC	Urinary corticosteroids (corticoid)
ZF	Zona fasciculata
ZG	Zona glomerulosa
ZR	Zona reticularis

PART I

LITERATURE REVIEW

CHAPTER I

THE ADRENAL CORTEX

1. EARLY HISTORY

The first report about the adrenal glands was by Bartholomaeus Eustachius in 1563 (Ibanez, 1952), under the name of "Glandulae Renibus Incumbentes". Since then several other names, such as "Glandulae Renales", "Capsulae Renales", and "Suprarenal Capsules" are recorded in the early literatures.

In 1803, Tilesius (Soffer, Dorfman and Gabrilove, 1961), reported the first recorded case with autopsy finding of tumour of the left adrenal in a four year old girl, who was enormously obese with a marked precocious development of the breasts. A similar case was described in 1811 by Cooke in another four year old obese girl with a tumour of the left adrenal; she died at the age of seven with enlarged external genitalia, extensive hirsutism of the genitalia and face, and low-pitched voice.

Significant advances in the knowledge of both morphology and function of the adrenal marked the latter

half of the 19th century. In 1855, Thomas Addison published his classical monograph "The Constitutional and Local Effects of Disease of the Suprarenal Capsules", in which he gave the characteristics of the adrenal deficiency disease which bears his name. In 1856, Brown-Sequard the French physiologist showed that dogs, cats and rabbits die twelve hours after bilateral adrenalectomy. This was the first proof that the adrenal glands were essential for life. In the same period, Claude Bernard, introduced the idea of internal secretion, that is the secretion into the circulation by an organ without ducts (Jones, 1957). Also in 1856, Vulpian reported the first significant observation on the adrenal medulla. He observed that when the adrenal medulla was moistened with dilute ferric chloride solution, a green coloration was produced. This observation led to the thought that the adrenal medulla secreted some unknown substance or substances which had a catechol nucleus but the exact significance and nature of which were not known at the time. Later, Oliver and Schaefer (1895) demonstrated that the adrenal medulla contained a potent pressor substance because of the remarkable rise in blood pressure following the injection of an extract of the adrenal medulla. They also showed that the active principle was produced in the medulla and not in the cortex. Because of the strong physiological

activity of the medullary substance, it was assumed until 1913 that death in adrenalectomized animals was due to lack of pressor activity. In 1913, Biedl demonstrated a functional division of the adrenal gland by showing that the removal of the cortex in dogs and rabbits, leaving the medulla intact, always resulted in death, while destruction of only the medulla had little effect. Animals would survive with only one-eighth of their adrenal tissue, provided it was cortical tissue. This was the first evidence that the adrenal cortex was essential for the maintenance of life. Later, he proposed the concept of an "endocrine integration" which, together with the notion of "internal secretion", promoted by Claude Bernard marks the dawn of modern endocrinology.

Between 1930 and 1940 a number of steroids were isolated from adrenal extracts by Reichstein, Kendall and Wintersteiner (Reichstein and Schoppee, 1943).

The first quantitative studies on the biochemical composition of the stimulated adrenal cortex were reported by Sayers' group in 1944 (Sayers, Sayers, Fry, White, Lewis and Long, 1944a, 1944b). These authors showed that the administration of adrenocorticotropic hormone (ACTH) to rats resulted in a decrease of both

the ascorbic acid and cholesterol concentration of the adrenals. Stress was shown to induce similar biochemical changes in normal rats (Sayers, Sayers, Lewis and Long 1947).

Thus, knowledge of the existence of the steroid hormones secreted by the adrenal cortices has been available for the past four decades and even though their chemical synthesis has also been achieved, a great gap exists between such detailed knowledge on the one hand, and our comparative ignorance of their mode of action, and even their functions under physiological conditions, on the other.

2. MORPHOLOGY OF THE ADRENAL GLAND

2.1. ANATOMY

Symington (1960) pointed out that the adrenals of various species can be classified into "non-fatty" and "fatty" types. The hamster and most herbivora fall in the non-fatty group, while other mammals, including man, fall in the fatty group.

The mouse adrenal glands consist of a pair of small ovoid structures situated one on either side of

the midline near the anterior pole of the kidney. The absolute weight of the adrenal increases rapidly in young mice, but the rate of growth declines with increasing age until it eventually ceases. This pattern is similar to that found in rats (Donaldson, 1924).

Sex differences in adrenal gland weight have been well recognized (Bourne and Jayne, 1961). Their weight in man is normally between five and seven grams, those of the female being consistently larger and more opaque due to the presence of lipid. The adrenals are smaller and dark red in males (Chai and Dickie, 1966).

2.2. HISTOLOGY

The adrenal glands are composed of two main parts: the medulla and the cortex, which are in fact two different glands. No direct functional relationship between them has yet been demonstrated in man. The cortex accounts for 80% of adrenal weight in the adult (Williams, 1962).

In man and most mammals, three zones are visible in the adrenal cortex, but in the mouse only two zones are clearly defined. Outwardly we find the zona glomerulosa (ZG) followed by the zona fasciculata (ZF),

which is the widest zone, and finally, the zona reticularis (ZR). No secretory nerves have been demonstrated in the adrenal cortex in contrast to the medulla. The ZG is a narrow zone consisting of small cells arranged in arches. The cells have relatively large nuclei, basophilic cytoplasm, and a rich capillary blood supply. The ZF is composed of long regular columns of cells separated by fine connective tissue septa bearing capillaries. The nuclei are vesicular and the cytoplasm acidophilic and foamy due to the presence of finely distributed lipid droplets.

While the three classical zones can be seen in many human glands, the ZG is by no means always prominent and may be seen in some part of a section and not in the others. The ZG has been shown to contain abundant mitochondria (Symington, 1962), variable lipid content (Carr, 1961), and greatest cell proliferation of the three zones (Ford and Young, 1963).

Symington (1962a) compared the histological structure of the adrenal cortex of a wide variety of species, laying considerable stress on the differences both in amount and in distribution of lipid. He pointed out, as did others, that there is no storage of hormone in the adrenal cortex in contradistinction to other

endocrine glands.

Ashworth, Race and Mollenhaver (1959) suggested that the major sites of hormone synthesis are the deep layers of the ZF and ZR since these areas are richer in mitochondria and vesicular cytoplasmic reticulum. The importance of the mitochondria in the synthesis of the adrenal cortical hormones is supported by independent biochemical studies demonstrating the association of enzyme systems with these bodies (Hayano, Saba, Dorfman and Hechter, 1956).

3. FUNCTIONS OF THE ADRENAL CORTEX

Reference to the work of Addison (1855), Brown-Sequard (1856), Oliver and Schaefer (1894-1895) and Biedl (1913) in regard to their classical discoveries on the function of the adrenal cortex has already been mentioned.

In 1932, Cushing described the syndrome now known under his name. A relatively frequent association of the basophil tumors of the anterior pituitary with adrenal cortical hyperplasia raised the question as to which came first. In 1933, Moehlig and Bates suggested that the primary lesions in Cushing's syndrome occurred in the adrenals. Two years later, Hare and his

co-workers reported a typical case of Cushing's syndrome due to a primary carcinoma of the adrenal cortex without an increase in the number of the basophil cells of the anterior pituitary. On the other hand, Oppenheimer and his co-workers cited 24 instances of basophilic pituitary adenomas without Cushing's syndrome (Soffer et al, 1961).

In 1943, Thompson and Eisenhardt came to the conclusion that the basic disorder of Cushing's syndrome is an excess of the adrenal cortical function with an altered pattern of secretion of the patient's own hypophysis.

In recent years, further advances in our knowledge of the mechanisms involved in the regulation of secretory activity of the adrenal cortex have been made: these include the isolation of corticotrophin-releasing factors (CRFs), the isolation of ACTH, the characterization of the intermediates and enzyme system concerned with corticosteroid (CS) synthesis, and the discovery of agents which block the CS synthesis.

3.1. THE EFFECTS OF ADRENALECTOMY (ADREX)

Many workers have demonstrated the rapidly fatal outcome of the adrex in laboratory animals. Many

factors determine the period of survival of animals following adrex. Certain animals (e.g., the mouse, rat, goat and frog) survive adrex much longer than others (e.g., the dog or guinea pig), due to their greater ability to withstand injuries in general, which in turn may be due to a more extensive distribution of adrenocortical tissue outside of the gland itself.

Adrexed animals are extremely sensitive to trauma and hemorrhage; hence, the finesse of the operative technique employed in removing the glands is an important factor in determining the period of survival. Young animals survive a much shorter period than do adult animals (Grollman, 1941).

In the well-operated animal no obvious abnormality is noticed at first. The animal is alert, eats, drinks, the blood pressure and all the constituents of the blood are in normal concentrations. However, gradual weakness appears in the animal, the animal becomes apathetic, refuses food, its muscular movements become slow, the body temperature falls, and muscular twitches and convulsions may occur. The respiration is at first rapid, then slow. Anuria is present, the pulse becomes feeble, the animal becomes comatose, and though the heart is still beating, respiratory paralysis sets in and leads to death.

Animals in adrenal cortical insufficiency are hypersensitive to many extraneous influences. They manifest an abnormal sensitivity to toxins, are very prone to infections, and frequently yield to influences which have only trivial effects in normal animals (Grollman, 1941).

In 1940 Swann wrote a comprehensive review of the early work concerning the physiological effects of adrex.

In summary, the effects of adrex are:

a) disturbance of the sodium (Na), potassium (K), chloride (Cl) ions and water balance,

b) increased excretion of Na and Cl ions as well as water while retention of K,

c) increase of urea content of blood,

d) disturbance of carbohydrate (CHO) metabolism with decrease in liver glycogen and decreased resistance to insulin,

e) reduction of resistance to various traumas such as cold, shock or injury.

Experimentally, Yates and Urquhart (1962) demonstrated that a combination of corticosterone (B),

cortisol (F) and aldosterone (Aldo) is sufficient to reverse the fatal effects of adrex.

3.2. ROLE OF THE ADRENAL CORTEX IN METABOLISM

3.2.1. CARBOHYDRATE METABOLISM

The hypoglycemic effect of adrenalectomy has been shown in the dog by Porges as early as 1910. Many other investigators since then have demonstrated the marked sensitivity of adrexed animals to insulin, and the reduced liver and muscle glycogen level in these animals. Simpson (1932) supplemented these observations by showing that patients with Addison's disease failed to show a rise in the blood sugar level comparable to that of normal individuals following the injection of a standard dose of epinephrine. The question arose as whether the CHO disturbances observed were not due primarily to the removal of the adrenal medulla. Patients with Addison's disease who have atrophy of the cortex, but with relatively intact medulla, nevertheless display the same characteristic disturbances in CHO metabolism as those who had extensive destruction of the adrenals due to tuberculosis. Long, Katzin and Fry in 1940 found that in both normal and adrexed fasting rats

and mice, the administration of ACE was followed not only by an increase in liver glycogen and blood glucose, but also increased the urinary nitrogen excretion.

During the early periods of investigation of the functions of the adrenal cortex, the relationship of the cortex to CHO metabolism was a source of great conflict between those groups who insisted that the CHO disturbances observed in adrexed animals were fundamentally related to the absence of the adrenal cortex, and their opponents who postulated that these disturbances were non-specific in character and rather related to the malnutrition so commonly present in the adrexed animals. Today there is no question concerning the fundamental role which the adrenal plays in CHO metabolism, inasmuch as the administration of glucocorticoids (GCCs) can reverse the effects of adrex on CHO metabolism.

3.2.2. ELECTROLYTE METABOLISM

3.2.2.1. Effect on Na

As early as 1927 Marine and Baumann showed that the Na content of the blood of cats decreased following bilateral adrex. In 1932, Rogoff stated that intravenous administration of physiological saline combined with

adrenocortical extracts are essential in treating patients with Addison's disease. By 1940, many investigators had shown an important role of the adrenal cortex in Na metabolism. In 1940, Wells and Kendall confirmed that the most potent adrenal hormone possessing an action causing renal retention of Na was DOC. It was, at that time the most potent of all known cortical hormones in maintaining the life of adrexed animals and in the control of certain phases of electrolyte metabolism. Dennis and Wood, in 1940 pointed out that the absorption of electrolyte from the lumen of the intestine is distinctly abnormal in adrexed animals. Stein and Wertheimer (1942) indicated that extrarenal defects may also be contributing to the impaired Na metabolism.

3.2.2.2. Effect on K

Baumann and Kurland (1927) and Hastings and Compere in 1931 reported that the removal of the adrenal glands was followed by a rise in serum K level.

In 1933 Loeb reported the retention of K ion in adrenal insufficiency even though this ion passes through the kidney with greater facility than either the Cl or Na ions. The author also reported a decrease in the Cl and bicarbonate (HCO_3) concentrations in the

blood. Winkler, Hoff and Smith in 1941, showed that not only was the adrexed animal exceedingly sensitive to administered K but that K salts given to the intact animal in quantities sufficient to raise the plasma concentrations to the level typical of terminal adrenal insufficiency would reproduce many of the symptoms of the adrexed animal, and might lead to death. Finally, the therapeutic value of a low K diet in the treatment of Addisonians or for the maintenance of adrexed animals has been repeatedly demonstrated by many authors.

3.2.2.3. Effect on H2O

In most animals, but not all, the altered electrolyte excretion which follows adrex is associated with a diuresis. Yet despite this water diuresis associated with Na loss, the ability of the kidney to excrete water shows deficiencies. If distilled water is administered by mouth, even in small doses, the diuretic response is far below normal (Rowntree and Snell, 1931; Levin, 1943) and the susceptibility to water intoxication is greatly increased (Rigler, 1935; Eversole, Gaunt and Kendall, 1942).

In 1949, Kendall wrote a review on electrolyte and water metabolism, and concluded that the metabolism

of Na, K and Cl was largely controlled by the adrenal cortex hormones. Kendall further stated that the adrenal cortex furnishes a mechanism to the body, which modifies the transfer of Na, K and Cl through cellular structures. Hormones of the adrenal cortex modify the transfer of electrolytes in the kidney and permit this organ to perform work to overcome the direction of the ionic changes which occur in the absence of the adrenal cortex.

The salt-regulating activity of the adrenal cortex controls (a) the acid-base balance of the body through its control of Na and Cl ions; (b) the body's degree of hydration and osmotic pressure through the control of the extracellular fluid and (c) muscle irritability through its control of K ions.

3.2.2.4. Aldosterone

In 1951, Bush applied a relatively new technique to paper chromatography and isolated concentrated adrenal extracts, which had strong Na retaining effect much greater than could be accounted for the corticosteroids known up to that time. They endeavoured to characterize the unknown compound, and finally Simpson and Tait in 1954 reported the chemical identification

of the aldosterone as delta^{4}-pregnene-11 beta, 21-diol-3, 20-dione-18-al.

Aldosterone promotes sodium retention and in this respect is 25 to 120 times as active as DOC in comparable amounts. It exerts its effects in very small quantities as compared with other known adrenal steroids. By 1960, evidence strongly suggested that the renin-angiotensin system (R.A.S.) might be the primary regulatory mechanism for Aldo secretion. During the past few years a large number of reports have provided convincing evidence that the R.A.S., plasma electrolyte concentrations, and ACTH influence the rate of secretion of Aldo. Renin is an enzyme produced by the juxtaglomerular apparatus in the kidney. It interacts with plasma alpha-2 globulin fraction, which contains renin substrate, to form angiotensin-I. The angiotensin-I is converted by an activating enzyme to angiotensin-II which is the active material responsible for elevation of blood pressure.

The role of the renin-angiotensin system in the control of aldosterone secretion has been demonstrated by Genest, Nowaczynski, Koiw, Sandor and Biron, 1961, and confirmed by several investigators. Exogenously administered angiotensin-II has been shown to increase aldosterone excretion and secretion in man and dog (Genest

et al., 1961, and Urquhart, Davis and Higgins, 1962). The existence of an inverse relationship between Na balance and the renin-angiotensin-aldosterone system has also been established (Genest, Veyrat, De Champlain, Boucher, Tremblay, Strong, Koiw and Marc-Aurèle, 1965; Fasciolo, De Vitio, Romero and Cucchi, 1964). Consequently, a hypothetical negative feedback mechanism was proposed, in which sodium loss leads to an increase in plasma renin activity (PRA). This, in turn, raises the concentration of angiotensin in the blood, stimulating the secretion of Aldo, and hence limits the extent of Na loss. Besides this proposed stimulus for the release of renin, an additional mechanism has been suggested, involving a decrease in the extracellular compartment or fraction thereof, and a reduction in renal artery pressure (Braun-Menendez, Fasciolo, Leloir, Munoz and Taquini, 1964). Increased PRA was found to occur in states of hypovolemia. However, conversely, increased extracellular fluid and a marked rise in plasma volume were also found in subjects with elevated levels of aldosterone and corticosterone (Bartter, Liddle, Duncan, Barber and Delea, 1956; Walter, Seldin and Burnett, 1955). Rosenthal, Boucher, Nowaczynski and Genest in 1968, investigated the effects of acute changes in plasma volume, renin activity,

and free aldosterone levels in healthy human subjects following fursemide administration, and suggested the following sequence of events:

a) the rapid activation of renin release secondary to the decrease in plasma volume,

b) leading to an increase in circulating amounts of angiotensin,

c) which results in a higher concentration of plasma aldosterone.

Aldosterone favors the excretion of potassium.

3.3. STEROIDS SECRETED BY ADRENAL CORTEX

There are about 50 steroids which have been isolated from adrenal cortices, but the number of new compounds which are discovered each year is increasing rapidly. Only a few of these steroids have been shown to be normally secreted into the blood stream. These include corticoids, aldosterone and sex steroids (Grant, 1960; Short, 1960). The remainder are intracellular intermediates. The adrenal CSs may be defined as steroids secreted by the adrenal cortex and possessing 21 carbon atoms and three or more oxygen atoms. The corticoids may

be further divided into those steroids possessing the characteristic delta 4-3 ketone in ring A, a group which are biologically active corticoids, and those with a reduced ring A structure. The compounds having glucocorticoidal action influence CHO, fat and protein metabolism.

Out of a long list of CSs discovered so far, only a few show predominantly glucocorticoidal or mineralo-corticoidal activity. The GCCs include corticosterone, 11-dehydro-corticosterone (A), 17 a-OH-corticosterone, cortisol, 11-dehydro-cortisol, 11-deoxycortisol (S), 11-deoxy-corticosterone (DOC), and the most potent mineralo-corticoid is aldosterone. Cortisol and cortisone exercise an effect mainly on CHO metabolism and to a much lesser extent on electrolytes. Their salt-retaining effects which are by no means a consistent phenomenon, are approximately 1/30 to 1/50 those of DOC which is itself about 25 to 120 times less potent than aldosterone in this regard (Jones, 1957).

The available experimental data suggest that these steroids stimulate gluconeogenesis from proteins and possibly from fats. In addition, they inhibit some phase of CHO utilization. Intensive stimulation

of the adrenal cortex in man with ACTH is followed by a fairly considerable increase in the urinary excretion of various amino acids (Roberts, 1952). The increase in the blood concentration of amino acids permits their greater diversion for gluconeogenesis.

As mentioned above, the most active mineralo-corticoid is aldosterone. Until the isolation of the aldosterone by Simpson et al, (1954) the most active mineralo-corticoid recognized up to that date was DOC. Progesterone, pregnenolone and 17-OH- pregnenolone have also been isolated.

The adrenal cortex also produces C-19 steroids such as delta 4-androstenedione (ASD) and its 11-B-OH and 11-keto derivatives, and dehydroepiandrosterone (DHEA) which have been isolated.

The synthesis of estrogens (C-18) by the adrenal is much less clear than that for androgens.

3.4. FUNCTIONAL ZONATION OF THE ADRENAL CORTEX

The functional zonation of the adrenal cortex has been established beyond any doubt in most laboratory animals and in man. Swann in 1940 reported that in hypophysectomized animals the ZG portion of the

adrenal cortex was not greatly altered, whereas the ZF and ZR rapidly atrophied in the absence of the pituitary; therefore, functional zonation concept of the adrenal cortex was put forward. Swann further stated that the ZG is responsible for electrolyte metabolism, ZF and ZR are responsible primarily for CHO and protein metabolism.

The functional zonation of the adrenal cortex later on was confirmed by many authors: Sarason (1943), and Deane, Greep and Shaw (1946, 1948). Bergner and Deane (1948) reported that ACTH has very little or no glomerulotrophic activity.

Ayres, Gould, Simpson and Tait in 1956 found that cortisol was produced in ZF, aldosterone in the ZG, and corticosterone in both zones. Giroud, Stachenko and Venning (1956) established that only glomerulosa tissue produces aldosterone, while the ZF and ZR produce mainly corticosterone but a small amount of corticosterone is also produced by ZG. Symington (1960) considered the ZF merely as a storage zone for steroid precursors and that the ZR is the actual site of C-21 steroids production with the exception of aldosterone.

4. THE EFFECTS OF STRESS ON THE ADRENAL CORTEX

In 1936, Selye reported that the organism responds in a stereotypical manner to a variety of widely different agents, such as infections, intoxications, trauma, nervous strain, heat, cold, muscular fatigue or X-irradiation. The specific actions of all these agents were quite different. Their only common feature was that they place the body in a state of general (systemic) stress. Selye in the same year put forward the concept of "General Adaptation Syndrome" which postulates that, when subjected to stress, an animal, besides undergoing adaptive changes specific to that particular stress, also undertakes a non-specific defense which is mediated by the pituitary-adrenal system. According to Selye, the pituitary-adrenal reaction is believed to be a defense or resistance mechanism common to all types of stress and to consist of four phases: shock, counter-shock, resistance, exhaustion, terminating in death should the stress be sufficiently severe. Adrenalectomy or hypophysectomy (hypox) affects only the resistance stage by considerably shortening it, but does not influence the shock and counter-shock phases (Selye, 1946).

It is quite well established now that the anterior lobe of the pituitary gland is the site of

formation, storage and release of ACTH which activates the adrenal cortex. The anterior pituitary usually has large stores of its hormones, including ACTH. In response to stress, there is a release of ACTH from storage, as shown by a fall in ACTH content of the pituitary gland immediately after stress (Rochefort, Rosenberger and Saffran, 1959). Under normal conditions, the adrenal cortex contains only minor amounts of the corticoids, but after stress and accompanying release of ACTH, the adrenal tissue contains higher concentrations of corticoids (Holzbauer, 1957). The depleted pituitary stores of ACTH are quickly restored after stress, suggesting that its synthesis is accelerated (Rochefort et al, 1959).

The pituitary is not essential to maintain a minimal secretion of cortical hormones as shown by the ability of hypoxed animals to survive a longer period than adrexed animals (Jones, 1954) which may not survive more than a few days after the operation (Firror and Grollman, 1933). Adrexed animals have diminished resistance to stress (Swingle and Remington, 1944; Sayers, 1950). Administration of ACE increases the resistance of hypoxed animals to stress (Baird, Cloney and Albright, 1933; Tyslowitz and Astwood, 1942).

In 1959 and 1960 Symington reported that the differences between the ZF, ZR zones disappear under stress conditions in human subjects. The ZR is the site of the production of CSs (except aldosterone), androgens and possibly estrogen hormones, and the clear cells of the ZF are a storage zone for steroid precursors. Under stress conditions, endogenous ACTH is liberated, the steroid precursors in the cells of ZF nearest the ZR are used for steroids synthesis, and when this results, morphological, histochemical and hydroxylating enzymatic changes occur in the cortex (Symington, 1962a).

4.1. ADENOHYPOPHYSEAL-ADRENOCORTICAL RELATIONSHIPS

In 1927 Smith demonstrated a role of the anterior pituitary in regulating the activity of the adrenal cortex. He reported rapid adrenocortical atrophy following hypox in the rat. He further demonstrated that substitution therapy, consisting of daily homotransplants of fresh pituitary tissues, could reverse the degenerative changes observed in the adrenal cortex after hypox. Later, Smith (1930) and Houssay and Sammartino (1933a) reported that the atrophy is limited essentially to the cortex, the medulla remaining unaffected. The atrophic process begins in the ZR and

eventually involves the ZF and to a much lesser extent, the ZG. When the process is complete, the cells are small and distorted, and the ZR is unrecognizable, while the ZF has completely lost its cord-like arrangement of cells. In 1949, Greep and Deane observed a great depletion of the lipids from the ZF in the hypoxed rat, while the ZG remained uninfluenced. He concluded that the ZG in the rat is capable of functioning independently. In the intact animal, the removal of one adrenal is promptly followed by a compensatory increase in the size of the cortex of the remaining adrenal. This phenomenon does not occur in the hypoxed animal. If ACTH is administered to such an animal, the usual hypertrophy of the remaining adrenal will ensue.

Sayers in 1950 suggested that ACTH secretion by the pituitary varies inversely with the concentration of circulating CSs. The hypothesis is based on the assumption that under condition of stress, there is an increased peripheral utilization of cortical hormone. The drop in concentration stimulates the adenohypophysis to discharge ACTH and brings the blood level of CSs back towards the initial level.

4.1.1. THE EFFECTS OF ACTH ON THE ADRENAL CORTEX

Of the many effects of ACTH on the adrenal cortex, it has been difficult to decide which effects are primary and which are secondary consequences of ACTH action. ACTH can increase the synthesis of active phosphorylase (an effect mediated by increased concentrations of a co-factor, adenosine 3' 5' - phosphate) in the adrenal cortex and this may have great significance because these enzymatic changes might provide a mechanism for increased steroid synthesis.

There is some evidence that the ACTH steroidogenic effect acts between cholesterol and delta 5-pregnenolone to progestrone (Kass, Hechter, Macchi and Moon, 1954).

The influence of ACTH in the regulation of aldosterone has not been as clear-cut as its role in the control of glucocorticoid production. It appears that the influence of the pituitary on aldosterone secretion by the adrenal cortex is less marked than its control over the production of glucocorticoid. In brief the effects of the ACTH on adrenal cortex are:

a) increased formation of pregnenolone from cholesterol

b) depletion of ascorbic acid

c) depletion of cholesterol

d) increased content of adenosine 3', 5' -mono-phosphate

e) depletion of glycogen

f) activation of carbohydrate metabolism

g) activation of phosphate metabolism

h) activation of nucleic acid metabolism

i) increased cellular activity

j) increased adrenal weight

4.1.2. EFFECTS OF HYPOTHALAMIC-HYPOPHYSEAL CONTROL ON THE ADRENAL CORTEX

It is now clear that although the CRF activity is present mainly in the median eminence (Matsuda, 1964), it is also present throughout the whole tuber cinereum and infundibular process of the hypothalamus. Stimulation of the hypothalamus in or close to the median eminence causes ACTH release (Mason, 1958), a response that is not prevented by pre-treatment with CS. This observation suggests that the part of the hypothalamus immediately involved in CRF release may not be CS-sensitive. At present it seems that the CS-sensitive element of the adrenal cortical feedback system probably lies in the medial, basal hypothalamus,

septum and rostral midbrain in rats and that the final step involved in CRF-release is not CS-sensitive.

It has been proposed that ACTH inhibits its own release by a negative-feedback action on the brain or pituitary (Kitay, Holub and Jailer, 1959).

5. SUMMARY

The acquisition of knowledge of the structure and function of the adrenal cortex is a remarkable chapter in the development of endocrinology. This rather minute organ has been shown to be essential for life particularly under conditions of stress and to have a wide variety of functions. Thus it produces profound effects on carbohydrate and protein metabolism and plays a central role in the control of the metabolism of sodium and potassium. Moreover, it plays a role in the development of secondary sex characteristics, especially under pathological conditions. About 50 steroids have been isolated from this gland and the number of new compounds discovered each year is increasing. However, only a few of these steroids have been shown to be secreted into the blood stream under normal conditions and these are the ones that have the effects just mentioned.

Finally, even though the existence of these steroids has been known now for several decades and their chemical synthesis has been achieved, yet a great gap exists between such detailed knowledge on the one hand and our comparative ignorance of their mode of action on the other. In short, much remains to be learned in this area.

CHAPTER II

THERMOREGULATION

1. HOMEOSTASIS

Many physiologists have been impressed by the self-regulating, negative feedback systems which serve to maintain the constancy of the internal environment of living organisms. This is termed "homeostasis". But homeostasis is not limited to animals; it is applicable to all living things. Engineers have long used such systems. The principle may even be applicable in sociology, economics and ecology.

The term "homeostasis" was first defined by the American physiologist, Walter B. Cannon (1939) as "a tendency to uniformity or stability in the normal body states (internal environment or fluid matrix) of the organism". Cannon paid homage to the German physiologist, Pflüger (1877), and the Belgian physiologist, Fredericq (1885), as well as to Hippocrates for the generation of the concept. Thus, in 1877, Pflüger stated, "The cause of every need of a living being is also the cause of the satisfaction of the need". Fredericq, in 1885, declared, "The living being is an agency of such sort that each disturbing influence induces by itself the calling forth of compensatory activity to neutralize or repair

the disturbance". The higher in the scale of living beings, the more perfect and the more complicated do these regulatory agencies become. They tend to free the organism completely from the unfavorable influences and changes occurring in the environment. Cannon also quoted the French physiologist, Charles Richet, as stating: "The living being is stable. It must be so in order not to be destroyed, dissolved, or disintegrated by the colossal forces, often adverse, which surround it. By apparent contradiction it maintains its stability only if it is excitable and capable of modifying itself according to external stimuli and adjusting its response to the stimulation. In a sense it is stable because it is modifiable - the slight instability is the necessary condition for the true stability of the organism." Cannon, in the French translation of his book, The Wisdom of the Body, stated: "The central idea of this book, 'the stability of the inner medium of the organism in higher vertebrates', is directly inspired by the precise view and deep understanding of the eminent French physiologist Claude Bernard". Thus, Cannon made it clear that Bernard deserves priority for emphasizing the role of the inner environment in the establishment and maintenance of steady states in the body.

There is now general concurrence that the term "homeostasis" was Cannon's, but the idea was Bernard's.

2. THERMOREGULATION IN BIRDS AND MAMMALS

Homeostasis is applicable to many systems in the organism (e.g. Ph of the blood, maintenance of the blood volume, control of organ size, the healing of wounds, control of food and water intake, etc.). Included here also is thermoregulation, the main subject of this thesis.

It is customary to name the maintenance of deep body temperature "homeothermy" while heterothermous (poikilothermous) animals are those in which deep body temperature follows the temperature of their environment. In a homeotherm, a regulated flow of heat determines body temperature. Hence the term "homeothermy" can mislead, since it may seem to mean "steady temperature", and in this way perpetuate the confusion between heat and temperature which has bedevilled the growth of animal energetics. Mendelsohn (1964) showed how the subject remained undeveloped until the concepts of heat and temperature were separated. Heat as a quantity of energy, and temperature as a measure of the ratio of heat content to heat capacity, are rarely separated in general usage, and are still confused even in some scientific contexts.

The most convenient definition of a "homeotherm" is an animal which maintains a steady internal temperature in a wide range of external temperatures. A heterothermous animal,

on the other hand, has nearly constant temperature only in an environment which has itself an almost unvarying temperature. Mammals and birds are homeotherms; other animals are poikilotherms. Homeothermic organisms are capable of maintaining a remarkably constant internal temperature even though the external temperature varies over a broad range. The combining form "Poikilo" means changeful or various. Thus, a poikilotherm has various internal temperatures dependent upon the external environment. They are often called cold-blooded animals. In the homeotherms we find that the deep body temperature, which is approximately the same for the core of the body (e.g. deep rectum, viscera, liver, brain) is regulated to constancy within a remarkably narrow range of temperature; human about 36.4 to 37.5 C with a diurnal rhythm.

One may wonder what advantage the homeotherms have as a result of possessing elaborate homeostatic mechanisms to control body temperature. The answer is simple- greater independence. Consider the frog, a poikilotherm. At the temperature extremes it cannot function. In the winter it becomes immobile, its body temperature being little different from that of the surroundings. To avoid freezing it seeks out the bottoms of lakes where the water remains above freezing. But even here it survives in a lethargic immobile state.

The better one's homeostatic processes, the greater is his independence of the environment, the more he can do and accomplish. A poignant example concerns man in his efforts to conquer space. To journey into space he must use a bulky space suit and a heavy capsule. These protect him against the extremes of temperature and pressure. How much freer, more independent, he would be if his own homeostatic mechanisms were adequate.

Another reason why such a constant temperature is desirable may be that the greater the complexity of the integration of the organism, the greater may be the need for constancy of temperature for efficient functioning. All chemical and physical reactions change their rate with the change of temperature, but the degree of acceleration with rising temperature is different for different reactions. Thus, in a complicated process involving the co-ordination of many individual reactions, a rise of temperature will not only speed the over-all rate but will alter the relative rates of the various reactions involved. The relative concentration of all the reactants will be markedly different when the new steady state, at a different temperature, is reached, and thus side reactions, connected with these reactants, will change their relative importance. A change of temperature therefore produces not only a change of the over-all rate of

metabolic and other biological processes, it also changes the qualitative character of these processes. Experience confirms that only a slight change in brain temperature, as in fever (even in a non-toxic, artificial fever) or hypothermia, does result in profound confusion in mental processes.

A confirmation of this idea that it is the complexity of organization that makes homeothermy necessary to an animal is the interesting fact that very young animals, in which the full co-ordination and integration of the nervous system is not yet fully developed, show an astonishing tolerance to changes of their body temperature (Adolph, 1950), together with a lack of temperature regulation. This tolerance is greater than that of the adult of the same species.

For an animal to maintain its body temperature in cold environments, its heat production must be continuously at a high level. This imposes a restraint upon the freedom of the homeothermic animal, which partly removes the advantages of emancipation from the thermal environment. A sustained high level of heat production, demands that the animal consume more food and thus more time and energy must be spent in obtaining it. In this sense there is a considerable cost to pay for homeothermy.

Recent reviews of thermoregulation have been published by Hardy (1961), Von Euler (1961) and Bligh (1966).

3. RESPONSES TO COLD

Special cases excepted, the outcome of a homeotherm's struggle against cold depends on the relation between three quantities: heat production, thermal insulation, and the temperature difference between the animal's core and the environment. The three main sorts of outcome of exposure to cold-metabolic, insulative (Hart, 1963, 1964a, b), and hypothermic (Hammel, 1964) - reflect these primary factors.

To regulate temperature, there must be sensing devices; in living organisms these are called receptors. In homeotherms, there are peripheral receptors (located in the skin) and central receptors (in the brain). One set of skin receptors responds progressively more rapidly as temperatures increase; another set responds more rapidly as temperatures decrease. Thus, sensing devices exist that, together, selectively function over a broad range of temperatures. Such a dual set of receptors also exists in the brain, more particularly in the hypothalamus. The skin receptors activate the nerves and, as a result, messages, in the form of electrical impulses, travel to the brain. These messages not only serve

to appraise the individual of the external temperature so that he can take voluntary action, but they also set into operation a sequence of nervous and metabolic events via receptors in the hypothalamus, which opposes the change in external temperature, thereby maintaining the constancy of the internal temperature.

The exposure of a homeotherm to the cold produces marked changes some of which involve the organism as a whole while others involve only certain organ systems, specific tissues and certain pathways of intermediary metabolism. All these changes are co-ordinated and directed towards the aim of helping the animal to survive the cold by maintaining the constancy of the core temperature through a regulation of heat production and heat flow. These changes will now be discussed.

3.1. BEHAVIOURAL CHANGES

A brief exposure to the cold requires only a temporary increase in heat production or thermal insulation; the latter may be brought about by nest-making by species that ordinarily do not make nests in the warm. Moreover, nest-making species make better nests during cold exposure, and select warm surfaces to sit on. Huddling by species ranging from mice to pigs can be an important means of

conserving heat. Efficient nest building may require a gradual learning from experience. Rats and pigs can learn to push a switch to turn on a source of heat. Mammals are less active in a cold than in a warm environment especially before cold adaptation is complete. Later, their activity increases. Many animals adopt a huddled posture to diminish heat loss due to radiation.

3.2. APPETITE AND FOOD INTAKE

In a cold environment, the appetite is increased. More food is taken in and metabolized, and thus more heat is generated. In addition, fat deposits result, and they serve as insulation. But intake is not adjusted to brief fluctuation of temperature. Cold-adapted animals probably also utilize their food more efficiently. The optimum diet for severe cold is still under investigation.

3.3. GROWTH

Long-lasting exposure entails structural changes as part of a chronic adaptation which can occur only if there is plenty of food. Heat conservation is aided by an increase in body weight in a cold environment, but such an increase rarely, if ever, occurs in an individual mammal on exposure to cold. Laboratory mammals usually lose weight on exposure to about 4°C,

but young ones may later restore it. Exposure to cold in early life may result in permanent stunting, but later exposure can have an opposite effect. Selection for resistance to cold over several generations, can result in a genetically determined increase in body weight.

Body shape may be influenced by the temperature of the environment during growth. The appendages, especially the tails of rodents, are shortened by cold. This serves to diminish heat loss, a desirable condition in the cold.

Adaptation to cold is accompanied, at least in laboratory animals, by the enlargement of organs that do more work, namely, heart, gastro-intestinal tract, liver, kidneys, thyroid and adrenal glands. In addition, hair usually grows longer during prolonged exposure.

3.4. BODY COMPOSITION

On sudden exposure to cold, there is a marked loss of adipose tissue and there may be loss of muscle mass. These can sometimes be made up after shivering has ceased, but living in a cold environment may entirely prevent the deposition of the amount of fat present in a warm environment. "Brown Fat" is a special type of adipose tissue, even more liable to loss than white fat, and produces much

heat on sudden exposure to cold. It is important in young mammals. Both types of adipose tissue undergo enzymic changes, evidently adaptive, during prolonged exposure.

Other chemical changes in cold-adapted small mammals include an increase in the proportion of water, and a decline in nitrogen, calcium and collagen.

3.5 REPRODUCTION

Ususally, mammals stop breeding in the cold season. This may be due to the shortage of food, rather than low temperature. Even laboratory mice, given excess food, and bedding, can breed in an environment at -3°C. Barnett and Coleman (1959) and Perrault and Dugal (1965) have suggested that the effects of cold and food shortage are often similar. Perrault and Dugal, in a study of testicular function in cold and shortage of food, distinguish a systemic action from a specific endocrine effect.

There is little information on the effects of cold on the reproductive powers of the male, most of the work concerns females. The general effect of a cold environment on female reproductive processes is to slow them down. The vaginae of random-bred mice, born in a room kept at 5°C, open later than those of controls at 21°C (Biggers, Ashoub, McLaren and Michie, 1958).

Low environmental temperature lengthens the oestrous cycle, and postpone the birth of the first litter in mice. The effect of cold on the reproductive performance depends on whether the animals have been transferred to a cold environment, or reared there. Mice transferred from 21°C to -3°C (with bedding) as young adults are less fertile; but their offspring are usually less fertile still. The third generation may, however, partly recover. The full scope of such maternal effects has still to be determined, but there is evidence of the possibility of a non-genetical, cumulative adaptation to cold over several generations.

3.6. ONTOGENY

Small mammals, and some large ones, are virtually heterothermous at birth, but some newborn artiodactyls, at least, are homeothermous. The newborn laboratory rat has some capacity to increase heat production if the outside temperature falls slightly, especially just after a meal; and the guinea-pig, cavia, has still greater powers of thermoregulation. Larger mammals respond more effectively; the hairless piglet, sus, can shiver virtually at birth. There is a rapid change towards the adult form of homeothermy during the first days or weeks. Newborn mammals can survive degrees of hypothermia which would kill adults.

3.7. HIBERNATION

Usually, if the thermal demand of the environment exceeds for long the temperature-maintaining ability of the organism, the animal dies. But there are part-time homeotherms, such as the humming-birds, Trochilidae (Lasiewski, 1963), which develop a regular nocturnal hypothermia; this is an energy-saving process in animals that need a high metabolic rate to maintain their body temperature. Similarly, seasonal hibernation, with its accompanying hypothermia, is a means of evading the consequences of food shortage in winter.

The entire cycle of hibernation is under precise physiological control. The preparation for hibernation takes place in various species and includes involution of endocrine glands, fattening, desaturation of depot fat and storage of food. Reductions in oxygen consumption, respiratory rate, and heart rate occur before a decline in body temperature as the animal enters the hibernating state. Animals may rewarm transiently as they enter hibernation. As the heart slows, peripheral resistance increases, thus keeping the blood pressure at reasonable levels. Although serum magnesium is high in mammals during hibernation (Riedesel, 1957), no other marked biochemical change has been universally noted. During hibernation mammals respond to dangerously low ambient temperatures by increasing their metabolic rate. They are sensitive to high

inspired CO_2 or low O_2. They are poised to wake naturally from hibernation or if physically disturbed. During arousal, oxygen consumption and heart rate may increase one hundred-fold, and the warming process is confined to the anterior portion of the body by differential vasoconstriction until the final phase of arousal.

3.8. HEAT PRODUCTION

Man, at rest under basal conditions, produces about 70 Calories per hour. But, heat production can be varied considerably. As the air temperature surrounding a nude subject falls below 32°C, the heat production progressively increases up to about 5 times the basal level as the air temperature approaches the freezing point. It can be seen further (Fig. 1) that at very high temperatures the heat production also increases. This, however, is not a homeostatic mechanism. Rather it represents a failure of homeostasis. At these high temperatures the air-conditioning system is overwhelmed; the internal temperature rises, and because of the heat, the cellular chemical processes are augmented. The rate of any chemical reaction is a function of heat; the higher the temperature, the faster the response. It is a direct effect of heat on chemical reactions. The increase in metabolism as the external temperature falls is

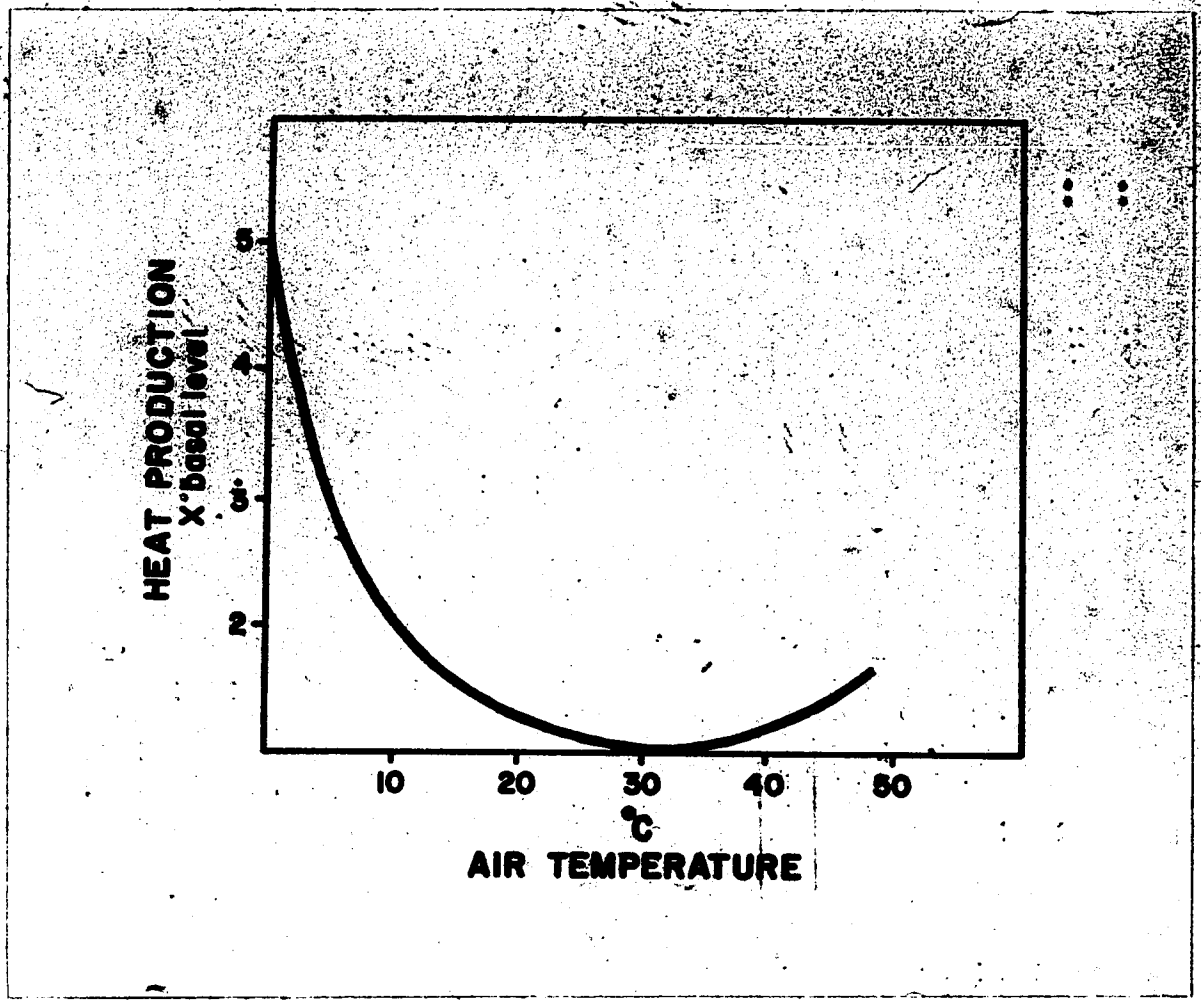

Fig. 1. Relationship of the heat production to air in man.

The increase in heat production below 32°C is a homeostatic response. The rise above 32°C is a direct effect of temperature on intracellular chemical processes (Langley, 1965).

an indirect effect. It is the indirect response that is important to homeostasis.

During exposure to cold the normal body temperature of the homeotherm is maintained, or the animal fails to survive. Consequently, heat loss must be decreased or heat production increased in order to maintain the balance. Several mechanisms may act to bring about a decreased heat loss. By far the most important, especially during short periods of exposure, is the peripheral vasoconstriction which reduces transference of heat from deep body tissues to the surface and in effect increase the insulating value of subcutaneous tissues. The increase in heat production, on the other hand, can be brought about by two means. The first or physical method consists of gross muscular activity and shivering.

3.8.1. THERMOGENESIS DUE TO INCREASED MUSCULAR ACTIVITY (SHIVERING, ETC.)

One way metabolic processes can be increased as the external temperature falls is by increased muscle activity. A series of chemical reactions takes place each time a muscle contracts and a by-product of those reactions is heat. Muscle contraction can be voluntary or involuntary. Involuntary muscle contraction caused by a drop in temperature is called shivering. Small groups of muscles contract asynchronously or

in opposition to each other so that very little or no movement of the parts of the body normally controlled by those muscles takes place. As was noted in Fig. 1, heat produced by violent shivering can equal five times the basal level.

In 1935 Denny-Brown, Gaylor and Uprus concluded that the frequency of the shivering tremor was 6 c/sec. in human subjects. They also concluded that the smaller muscles contracted more rapidly than the larger ones and the movement of the jaw and shoulder joints was slower than the distal limb joint tremors. They also determined that the discharges of the groups of fibers innerated by single motor neurons, conventionally termed neuromotor units, fuse together during intense shivering into what is termed a "grouped voltage" discharge. Additionally, it is observed that shivering is more intense during inspiration than between breaths, (Burton, 1937).

If shivering does not produce heat fast enough to offset the loss, another recourse left is to produce heat even faster by vigorous voluntary muscle contraction.

3.8.1.1. Physiological Stimuli of Shivering

In 1860 Liebermeister proposed that the stimulus to evoke shivering was a falling skin temperature. In 1892 Richet demonstrated that shivering could be evoked by either a

falling skin temperature or brain temperature or a combination of both. Richet did not measure the brain temperature directly but assumed it paralleled the blood temperature of deep body structures. This has recently and correctly been challenged by Benzinger (1960), but it is valid to the extent of the gross alterations of deep body temperature that Richet and subsequent workers have induced experimentally.

It seems certain that shivering can be evoked by a falling skin temperature with little or no change in brain temperature. This has been shown in unanesthetized humans and cats and anesthetized cats and dogs by different workers, and relatively recently by Hensel in 1961.

It appears that little has been learned of the central neurogenesis of shivering by the previous experiments concerning whether the physiological stimulus to evoke it is falling skin and/or brain temperature. It seems that it is essential first to determine the location of the central nervous cells whose discharge is related to changing skin temperature, others whose discharge is related to the reception of changing blood temperature, and those whose discharge is related to the activation of shivering. When this information is known, it will be possible to study the interaction between these three sets of neurons at different levels of brain and

skin temperature and hence learn far more about the physiological stimulus that evokes shivering.

3.8.2. NON-SHIVERING THERMOGENESIS

The second or chemical method of increasing heat production is mediated by increased hormonal secretion, enzymatic adaptations and other biochemical modifications which increase heat production independently of muscular contraction. The catecholamines play a central role here (Carlson, 1960) and their effects in the response to cold stress can be ascribed to their vasoconstrictor action as well as to their stimulating effect on metabolism.

The occurrence of non-shivering thermogenesis in the cold-acclimated animal has been well demonstrated, at least in the rat (Sellers, Scott and Thomas, 1954; Hart, Heroux and Depocas, 1956). Such an increase in non-shivering thermogenesis may be due either to a greater release of catecholamines or to a greater sensitivity of acclimated animals to these hormones or to both mechanisms. A greater sensitivity of cold-acclimated rats to the calorigenic effect of adrenaline has been first observed by Ring (1942) and confirmed later by other workers. Hsieh, Carlson and Gray (1957) also reported a striking calorigenic action of noradrenaline in cold-acclimated rats, which was confirmed by Depocas (1960a, b).

Although the calorigenic effects of both adrenaline and noradrenaline are intensified in cold-acclimated rats, the acceptance of noradrenaline as the mediator of non-shivering thermogenesis rests on the following observations (Depocas, 1961). Adrenal demedullation lowers the increase in oxygen consumption of the curarized cold-acclimated rat at 6°C (Cottle and Carlson, 1956), but does not abolish it as does previous injection of sympatholytic and ganglion-blocking agents (Hsieh, Carlson and Gray, 1957). Noradrenaline is more effective than adrenaline in preventing the fall in oxygen consumption caused by hexamethonium in curarized cold-acclimated rats (Hsieh, Carlson, 1957). Cold acclimated rats show a greater sensitivity to injected noradrenaline than adrenaline (Hsieh and Carlson, 1957). There is a relationship between the calorigenic response and the dose of noradrenaline administered and also the time of exposure to cold (Depocas, 1960a, b). A striking inverse relation exists between the increase in the metabolic response to noradrenaline infusion during acclimation to cold (Depocas, 1960a,b) and decrease in muscle electrical activity (Hart, Heroux and Depocas, 1956), suggesting a substitution of non-shivering thermogenesis under the control of noradrenaline for the shivering heat production. Neither cold exposure nor noradrenaline induces marked hyperglycemia as would be obtained by similar doses of adrenaline (Hsieh and Carlson, 1957).

It can be concluded that, if the role of catecholamines in the immediate response to cold exposure is at the present not clear, a good deal of evidence supports the idea that the non-shivering heat production, associated especially with acclimation to cold, is mediated through the release of noradrenaline, presumably from the adrenergic nerve endings.

3.8.3. THE RELATIONSHIP OF SHIVERING TO NON-SHIVERING THERMOGENESIS

Claude Bernard (1876) and other investigators of the latter half of the 19th century, mainly Rohrig and Zuntz (1871), believed that cold-induced increases in heat production were mainly a result of increased muscular activity, including shivering.

Rohrig and Zuntz in 1871 paralyzed the muscles of various mammals with curare and showed that in all cases there was a subsequent decline in body temperature accompanied by a fall in the oxygen consumption rate. In homeotherms chronically exposed to cold, increases in oxygen rate can occur in the absence of shivering or any other form of muscular activity. The evidence for such non-shivering thermogenesis has been reviewed by Morin (1946), Hart (1958) and Smith and Hoijer (1962). However, in homeotherms not chronically exposed

to cold there is little evidence of non-shivering thermogenesis. In humans, Cannon, Querido, Britton and Bright (1926-27) showed that there is a cold-induced increase in metabolism of 16.5% in the absence of shivering, in contrast to a 90% increase during shivering. Davis and Meyer (1955) concluded that the cold-induced 100% increase in the oxygen consumption rate of shivering rats can be partitioned into 40% due to non-shivering thermogenesis and 60% due to shivering. Cottle and Carlson (1956) were able to show only a 10% to 25% cold-induced increase in the oxygen consumption rate of rats in which all the somatic musculature had been paralyzed. Shivering is evidently the most potent mechanism available to the homeotherm for increasing oxygen consumption rate in the face of a sudden drop in the environmental temperature. This increase is at least 10 to 20 fold that which could be accomplished in the absence of muscular activity.

It is not well known why homeotherms do not respond to brief and sudden decreases in the environmental temperature by a vigorous physical activity that can elevate oxygen consumption to a far greater extent than can shivering (Robinson, Edwards and Dill, 1937). Hart and Heroux studied this problem in mice (Hart, 1950a, b), lemmings and rabbits (Hart and Heroux, 1955) and concluded that the heat produced by forced running was dissipated and not available for thermoregulation in cold environments. They found, associated with

an increased dissipation of heat, a decrease in body insulation and a fall in rectal temperature while the animals were running vigorously in a cold environment. It would thus appear that the advantage of shivering over that of vigorous exercise lies in the reduction of functional surface area, the shivering animal being in a huddled posture that reduces heat loss to the cold environment.

The increase in the oxygen consumption rate during shivering is somewhat variable. Fredericq (1882) modified the closed circuit spirometer and made a quite accurate determination of the oxygen consumption rate and rectal temperature while cooling humans, cats and rabbits. When shivering occurred, it elevated the oxygen consumption rate two - to four - fold above the resting non-shivering level, a fact which has been confirmed by König (1943) and Adolph and Molnar (1946) for man, and by Lim (1959) for dogs. Investigators in this field notice variances in elevated oxygen consumption rates accompanying shivering, but they seldom report or emphasize them in the literature on temperature regulation.

In summary, it appears that shivering is the most effective mechanism available to homeotherms in order to increase oxygen consumption rapidly in the face of a brief and sudden drop in the environmental temperature. The increase so

effected varies from two - to four - fold the resting non-shivering oxygen consumption rate.

3.8.4. TISSUE METABOLISM

Field, Belding and Martin in 1939, determined in vitro the metabolic rates of 20 major organs and tissues from the albino rat. By multiplying each by the weight of the respective organ, they obtained figures representing the rate of the various organs. The sum of these values was equal to 65% of the MR (28°C) of the animals used. Allowing an additional 25% for skeletal muscle tone, cardiac, respiratory and secretory activities, they accounted for 89%. Similar measurements by Martin and Fuhrman (1955) for mice and dogs gave figures of 72 and 89%, respectively. Using a technique in which tissues in flasks were exposed to oxygen were obtained directly rather than being immersed in saline, values of 100% for the rat (Huston and Martin, 1954).

Metabolic alterations which occur in tissues during adaptation to cold (5°C) have been studied in male albino rats by Cottle (1958). Metabolism at 5°C was found to increase gradually for 2 to 3 weeks. This was seen by measurements of heat loss, oxygen consumption, and food intake. After 2 weeks at 5°C, metabolism at 28°C was 20% greater than that of controls kept at 26°C. Metabolism of skeletal muscle, liver,

and kidney *in vitro* suggested that the increase in metabolic rate was not merely a reflection of the increased intrinsic metabolism of tissues.

Kleiber (1941,1947) and Weymouth, Field and Kleiber (1942) found that the *in vitro* metabolism of liver slices prepared from rats, rabbits, sheep, horses, and cows decreased in this order and to the same extent as the metabolism (BMR) per unit weight. They concluded that the factors which determine the metabolic rate *in situ* still control it on surviving tissues.

Other workers have concluded that the factors responsible for the metabolic rates of tissues *in situ* are not operative *in vitro*. Krebs in 1950 determined the Q_{O_2} (cc.02/gm. dry weight/hr.) for several tissues from nine mammalian species of various sizes. He found a somewhat lower Q_{O_2} for tissues from larger animals than for homologous tissues from smaller species, the Q_{O_2} of most tissues differed less with body weight than did the metabolism (BMR) of the whole animal.

The liver metabolism of rats after exposure to cold for 2 days or more measured *in vitro* is greater than that of controls kept at room temperature (Cottle, 1958). You and Sellers (1951) reported that liver slices from animals kept 1 year at 1.5°C had a Q_{O_2} 28% greater than slices from animals kept at 22°C. Clark, Chinn, Ellis, Pawel and Criscuolo in 1954

reported a 40% greater liver QO_2 and a 35% greater liver succinoxidase activity in young rats after 10 weeks at 4°C. Six month old rats were reported to have a 27% greater liver QO_2 after 10 days at 5°C. (Weiss, 1954). DesMarais (1953) indicated that liver slices from rats kept at 4°C for 2 to 8 days had a QO_2 12% greater than those from animals at 29°C.

An increase in the intrinsic metabolism of several other tissues as result of cold adaptation was shown by Weiss in 1954. Using young rats (100 to 150 gm.), he found that those kept for 10 to 26 days at 5°C compared to controls at room temperature had a 43% greater liver QO_2, 13% greater kidney QO_2, 23% greater heart QO_2 and 19% higher diaphragm QO_2. In 1956 Weiss reported that the QO_2 for skeletal muscle from young rats (100 gm. or less) was significantly greater for the animals which had been at 5°C for 10 days than for the young controls which had been kept in room temperature. In older rats (300 to 400 gm.), he demonstrated a decreased QO_2 after cold adaptation (Weiss, 1954). He suggested that the difference between the age groups was due to the greater insulation and fat accumulation of the older animals. He postulated that the greater insulation of the older group served to reduce effective exposure. He believed more severe cold exposure would have produced changes in other organs of the older animals. The additional changes in young rats were

probably related in some way to their high growth rate and energy turnover.

Weiss speculated that all tissues do not acclimatize at the same time. The liver is first to increase oxygen consumption in response to climatic stress, and other organs need a greater stimulus. A more reasonable explanation is that the prior increase in liver metabolism results from the demands on the liver, when an animal is exposed to cold, to supply increased amounts of metabolites to responding organs.

DesMarais (1955) reported that succinic dehydrogenase activity in rat skin was greater after 2 to 4 days at 4°C, than in the controls which were kept at room temperature. He suggested that the increased metabolism of the skin might contribute a consequential amount of heat to the animal in the cold. The increase could reflect the lower functional temperature of the skin.

3.8.5. INTERMEDIARY METABOLISM

3.8.5.1. Fat Metabolism

In 1949, Sellers and You presented the first unequivocal evidence that cold acclimation has a pronounced action on hepatic lipid metabolism. In these studies they

found that the feeding of a hypolipotropic diet of moderate fat content causes an excessive hepatic fat deposition in rats living at room temperature, but not in those living at 1.5°C (Sellers and You, 1949, 1952). Sellers and You called this phenomenon the pseudolipotropic action of cold exposure. Treadwell, Flick and Vahouny, in 1957 and 1958, obtained confirmatory evidence for this pseudolipotropic action of cold exposure. More striking is their finding that the exposure of rats with nutritionally-induced fatty livers to a cold environment tends to restore the liver lipids to normal, although the animals continue to ingest a hypolipotropic diet (Treadwell et al, 1958). In 1959 Masoro and Felts showed the complexity of the relationship between cold exposure and hepatic lipid levels in the fasting rat (Felts and Masoro, 1959). They concluded that the pseudolipotropic action of cold acclimation relates not only to hypolipotropic diets, but to other factors causing fatty livers as well.

Masoro, Cohen and Panagos in 1954, reported that cold stress greatly depresses hepatic lipogenesis. Later it became evident, however, that the liver is probably not the major site of lipogenesis in the mammalian organism. Favarger and Gerlach (1955) and Favarger (1955) reported on studies with intact mice and indicated that only 4% of the

lipogenesis of the mouse takes place in the liver and that most of the remaining fatty acid synthesis was carried out in the adipose tissue. Masoro, Porter and Patkin (1961) investigated the effect of cold stress on adipose tissue and discovered that cold stress, which almost abolishes hepatic lipogenesis, does not appreciably alter adipose tissue lipogenesis. In most other conditions studied (fasting, diabetes, insulin administration), the lipogenic activity of the liver and adipose tissue respond in a qualitatively identical manner (Hausberger, 1958; Winegrad and Renold, 1958). Milstein and Hausberger (1956) reported that, when fasted rats are made hypoglycemic by the administration of large doses of insulin, hepatic lipogenesis ceases completely, while adipose tissue lipogenesis is increased above the normal.

The loss of lipid of the brown adipose tissue during the initial exposure to cold of a warm acclimated rat was first reported by Selye and Timiras in 1949. At about the same time, the increase in the weight of brown adipose tissue of the rat on prolonged exposure to cold was described by Pagé and Babineau (1950). These authors observed an increase in wet weight which was due to increases both in dry material and water content; the total lipid content remained the same. In experiments done by Himms-Hagen (1965), the increase in wet weight was due to increases in all three components:

lipid content, nonlipid materials and water. She also showed that after 1.5 hours in the cold, the loss of lipid and gain of water is accompanied by a gain in protein. Presumably, the increase in protein content marks the start of the growth of the tissue. The nature of stimulus to the growth of tissue is uncertain. Possibly, the prolonged sympathetic stimulation is responsible. However, increased adrenal cortical activity or increased thyroid activity can also cause hypertrophy of the brown adipose tissue in warm-acclimated rats (Lachance and Pagé, 1953, 1955) and the activity of both of these glands is known to be increased in rats exposed to cold. The growth response of the brown adipose tissue is not specific to the stimulus of cold. It also occurs in rats stressed by other means such as forced exercise or spinal cord section (Lemonde and Timiras, 1951).

Pagé (1957) and Masoro (1963) demonstrated an increased lipid synthesis in brown adipose tissue of cold-acclimated rats. Similarly, following cold exposure, de Freitas (1967) has shown that the rate of synthesis of neutral glyceride glycerol was increased about 120 times in interscapular brown fat while only twice as much in liver and 4 times as much in carcass. On the other hand, the synthesis of neutral glyceride fatty acid was slightly increased in carcass and liver but somewhat decreased in the brown fat. At the present time, there is much interest in the role of brown fat in thermogenesis,

many being of the opinion that it has a special role in this process. Moreover, Patkin and Masoro (1964) demonstrated an unaltered fatty acid synthesis *in vivo* in brown or white adipose tissue of cold-acclimated rats in the cold. On the other hand, an increased capacity for fatty acid synthesis *in vitro* by white adipose tissue was observed in cold-acclimated rats (Patkin and Masoro, 1961) and hamsters (Baumber and Denyes, 1963). Finally, an unaltered capacity for fatty acid esterification *in vitro* by white adipose tissue was observed in cold-acclimated rats (Patkin and Masoro, 1961).

The postulate that the accelerated turnover of brown adipose tissue might be a major mechanism in non-shivering thermogenesis under the control of the sympathetic nervous system is in accord with the suggestion made by Smith and Roberts (1964) that brown adipose tissue is a major site of thermogenesis in the cold-acclimated rat exposed to cold, and with the suggestion made by Ball and Jungas (1961) that the triglyceride cycle of adipose tissue, particularly brown adipose tissue, might be utilized by homeothermic animals to generate heat for the maintenance of body temperature. It is known that the oxygen consumption of brown adipose tissue *in vitro* is increased by noradrenaline (Joel, Treble and Ball, 1964) and by adrenaline (Joel and Shackney, 1962). The

triglyceride cycle meets the requirement that "the heat producing mechanism be subject to rapid on-off control" (Smith and Roberts, 1964).

3.8.5.2. Carbohydrate Metabolism

When a small mammal such as a rat becomes acclimated to cold, certain alterations in the metabolism of glucose are observed. It has been reported that the intact acclimated rat forms liver glycogen from ingested or injected glucose at much greater rates than control animals (Pagé, Babineau and Lachance, 1955). Similar results have been obtained *in vitro* with diaphragm muscle from acute but not chronically exposed rats (Wertheimer and Bentor, 1957). Moreover, Hannon and Young (1959) have observed that fasting cold-acclimated rats maintain blood sugar levels as well as do fasting warm-acclimated rats. In addition, it has been shown that acclimated rats maintain higher fasting liver glycogen stores than controls (Masoro and Felts, 1959). Since fasting blood sugar levels are the same under the two environmental conditions, this would seem to indicate that cold exposure leads either to an augmented gluconeogenesis or to a 'sparing action' on carbohydrate stores. Existing data indicate that acclimation is associated with increased activity of various components of the tricarboxylic acid

cycle of liver and muscle tissue (Hannon, 1958, 1960).

It is generally agreed that the cold-acclimated rat has an augmented capacity both for glucose formation and for its utilization. This is not too surprising in view of the fact that these animals consume at least 50% more food (including carbohydrate) than non-exposed animals. However, the fate of this carbohydrate when taken up by the cells is still somewhat uncertain. One may probably assume that part of the augmented utilization includes an increased capacity for glycogen synthesis. On the other hand, one cannot safely assume that an increase in glucose utilization implies a corresponding increase in glucose oxidation. For instance, the possibility exists that a sizable portion of ingested and subsequently glycolyzed glucose might be diverted to amino acids and fats. If this were true, then it would be conceivable that during the post-absorptive period these animals could support their high tissue oxidative rates with substrates other than carbohydrates.

3.8.5.3. Protein Metabolism

As early as 1938 it was reported that protein metabolism was unaltered by cold exposure (Dontcheff and Schaeffer, 1938). Other reports, however, indicated the contrary, such as that published in 1950 by Hoberman, who

reported that amino acid and protein catabolism is accelerated in fasting cold-exposed rats. Williams, Schurr and Elvehjem, 1950), using fasting rats exposed to -5°C for 6 hours, reported a marked decrease in plasma proline followed by methionine, threonine, arginine and lysine. In contrast, the plasma levels of leucine, phenylalanine and valine were found to be markedly increased.

Mefferd, Hale and Martens (1958) found that cold-exposed rats excrete more alanine, valine, serine, threonine, glycine and glutamic acid than their warm counterparts.

Effect of cold exposure on two enzymes directly involved in the protein metabolism has been reported. Schayer (1960) found a marked increase of histidine decarboxylase in mice exposed to 2°C for six hours, and Vaughan, Vaughan and Klain (1962) found a higher activity of xanthine oxidase in the liver and kidney of rats exposed to 7°C for four weeks. Trapani and Campbell (1959) reported that the disappearance of passively administered antibody is more rapid in rabbits at -15°C than at 18°C, thus indicating faster protein turnover in the cold. From the work of Trapani (1960) it is apparent that cold-exposed animals exhibit changes in the electrophoretic distribution of the plasma proteins, an increase in the total

mass of circulating protein, an increase in protein turnover, and an apparent decrease in the immune response. Furthermore, Hannon and Young (1959) reported that one month's cold exposure of rats resulted in a significant decrease in plasma protein levels.

Excretion of nitrogenous compounds other than amino acids was also increased in the cold, regardless of whether food intake was equalized (Lathe and Peters, 1949; Ingle, Meeks and Humphrey, 1953) or whether the rats were allowed to feed ad libitum (Hannon and Young, 1959; Treichler and Mitchell, 1941; You, You and Sellers, 1950). Cold-exposed rats excrete more urea, allantoin, creatine, (Young and Cook, 1955; Mefferd, Hale and Martens, 1958; Hale and Mefferd, 1958) and creatinine (Treichler and Mitchell, 1941; Selye, 1946).

The salient points derived from studies utilizing immunological techniques and free-boundary electrophoretic analyses of the plasma proteins and which can be related to protein metabolism in the chronically cold-exposed animal are: changes in the electrophoretic distribution of the plasma proteins, an increase in the total mass of circulating protein, an increase in protein turnover, and an apparent decrease in the immune response. It is felt that the

activity of the thyroid may exert some influence on these parameters in the cold-exposed animal. Since it is difficult to ascribe the changes in protein metabolism to the cold exposure itself, it seems reasonable to attribute these alterations to the interplay of several factors including the hormonal alterations consequent to the stress of the cold exposure.

3.8.6. NEURO-ENDOCRINE RESPONSES TO COLD EXPOSURE

3.8.6.1. Hypothalamus

The thermoregulating system can be divided into sensing elements, integrating elements and effector elements. There is impressive evidence that thermoreceptive structures in the skin (sensing elements) play a part in thermoregulation. Thermal receptors for thermoregulation (integrating elements) occur in the preoptic region, and perhaps in other regions of the brain. The presence of sensing elements in the hypothalamus has been demonstrated by ablation techniques; and by selectively and locally heating and cooling the hypothalamus, it was shown that overall thermoregulatory responses could be elicited from this region. Hardy, Hellon and Sutherland (1964) have explored the preoptic region and the anterior and posterior hypothalamus as well as other formations with microelectrodes,

while locally changing the tissue temperature with thermodes. In conscious animals, similar manipulations resulted in substantial thermoregulatory responses to changes in thermode temperature. Such overall responses are absent in the anesthetized reparation.

Three basic types of neurons were found in the preoptic area. The most common type of unit was not responsive to its own temperature in terms of changes in firing rate ("temperature insensitive") unit. A second group responded to increases in local temperature with increases in firing rate ("warm sensitive") units. A third group responded to increases in local temperature with a reduction in firing rate ("cold sensitive") units. In contrast to many receptor structures, these central thermoreceptors had little or no rate sensitivity. Their response to a given temperature was independent of the rate of change of temperature. By using special dynamic techniques, it was possible to demonstrate that some neurons were not sensitive to their own local temperature but to local temperatures at some distance away. This is an indication of transmission and possibly integration in the anterior hypothalamus.

Recently, Wit and Wang (1967) have shown in cats an effect of peripheral whole body heating on firing rates in thermosensitive structures in the anterior hypothalamus. All

homeothermic species investigated have thermosensitive neurons in the preoptic anterior hypothalamic region. In another report, peripheral warm and cold stimulation has no effect on preoptic temperature sensitive and insensitive neurons (Murakami, 1967).

Hammel, Caldwell and Abrams (1967) found in a poikilotherm, the Australian blue-tongued skink, that whenever possible this lizard maintained a desirable internal temperature by behavioral adjustments. However, upon investigation, even this poikilotherm was found to have temperature sensitive neurons in the preoptic anterior hypothalamus. Hammel selected experimental conditions so that peripheral temperatures could be varied, and studied the integration of peripheral and central thermal signals. He concluded that the central and peripheral signals were basically linearly additive. Changes in peripheral input would be similar in effect to changes in the central "set point".

3.8.6.2. Pituitary Gland

Cold exposure of hypothermal animals induces a profound change in anterior pituitary function. The secretory alterations in the adenohypophysis reflect the strategic role of this gland in neuroendocrine integration. Through a unique anatomic and physiologic relationship with the hypothalamus and

by virtue of its trophic hormone regulation of target gland function, the anterior pituitary insures effective hormonal contribution to the cold acclimation process. The mechanisms basic to endocrine readjustment at low temperatures are by no means understood. Although the specific hypothalamic-hypophysial interactions have not been elucidated completely, there is convincing evidence that in acute exposure to cold, hypothalamic stimuli quickly facilitate significant shifts in trophic hormone secretion by the pituitary. Appropriate trophic hormone responses to cold, triggered and probably maintained by neural influences, insure adequate production and delivery of pertaining hormones to the circulation.

Among the pituitary trophic hormones, effect of cold on thyroid stimulating hormone (TSH) and ACTH has been studied by different investigators. Measurement of I^{131} release rate by the thyroid in rat and guinea pig reveals that the tempo of thyroid hormone secretion after 6-8 weeks of cold exposure is significantly increased in both species. The relatively slow release rate in the guinea pig at normal room temperatures was approximately doubled in the cold. In the female rat the normally rapid I^{131} release from the thyroid increased from a rate of 25% to about 40% per day (D'Angelo, 1960). Enhanced thyroid was definitely related to increased TSH output by the pituitary. D'Angelo also studied the guinea

pig's pituitary-adrenal system in cold, and reported that at 7°C for 7 weeks there was significant increase in adrenal-body weight ratios and in urinary excretion of corticoids while ascorbic acid concentration within adrenal remained normal. A rise in the level of corticoid excretion, noted within the first week of exposure, reached a peak value at the end of the fifth week. Just as with the thyroid's dependence on TSH, enhanced adrenal function was clearly referable to augmented ACTH secretion by the adenohypophysis. It was demonstrated in bio-assay experiments that ACTH concentration in the pituitary of the refrigerated guinea pig was increased 10-15 fold.

In brief, appropriate trophic hormone responses to cold, triggered and probably maintained by neural influences, insure adequate production and delivery of thyroid and adrenocortical hormones to the circulation. These target gland hormones in turn promote the cold acclimation process by influencing cellular metabolism in ways not yet precisely determined.

3.8.6.3. Thyroid Gland

Thyroid hormone by its nature appears to be related to the changes seen in small homeotherms upon chronic cold exposure. Morphological changes in the thyroid have

been seen in a number of species following prolonged cold exposure. These changes include an increase in gland weight (Sellers and You, 1950; Rand, Riggs and Talbot, 1952), increased vascularity (Kenyon, 1933), and decreased colloid within follicles (Wolf and Greep, 1937). Each of these changes is a recognized criterion of increased thyroid function.

There is some evidence that thyroid activity in rats reaches a maximum within about 3 weeks exposure to cold and returns to near normal condition after 6-10 weeks. Starr and Roskelley (1940) found the height of the follicular cells in thyroids from rats kept at 15°C reached a maximum in about 3 weeks and returned to near normal in about 6 weeks.

Heroux, Schönbaum and Des Marais (1959) did not find histological evidence of increased thyroid function in rats kept 3 months outdoors in winter conditions. These rats, on the other hand, had developed the same degree of cold resistance as animals kept in the cold under laboratory conditions, under which conditions there was an increased level of thyroid function.

Estimates of the amount of thyroid hormone secreted by rats have indicated those at 5°C produce about twice as much as those at 25°C.

The determination of thyroidal radioactivity at various periods after administration of radioactive iodine (I^{131}) is used widely as an assay of thyroid function. Uptake by the thyroids of rats kept 1-3 weeks at 4°C and determined 2 hours after injection of the isotope has been found to be greater than in rats kept at room temperature (Leblond, Gross, Peacock and Evans, 1944). After 6 weeks, 2-hour uptake was found similar to that of controls. This decrease in uptake appears to confirm the histological findings.

The increased thyroid activity in the cold is brought about through the release of thyroid stimulating hormone (TSH) by the adenohypophysis. Thyroids of hypophysectomized animals exposed to cold do not show evidence of increased thyroid secretion seen in intact animals (Knigge and Bierman, 1958). Basophil cells, believed responsible for the production of TSH, are numerous and are hypertrophied in pituitaries removed from cold-acclimated rats (Brolin, 1946). Finally, TSH is present in serum of cold-exposed animals at a higher level than in serum from controls, (Stevens, D'Angelo, Paschkis, Cantarow and Suderman, 1955).

Thyroid function is affected by the level of food intake. The rate of release of hormone during acclimation to cold is very much reduced by limited restriction of food.

Ring (1942) suggested that the increase thyroxine release in the cold brings about changes which enhance the calorigenic response to endogenous adrenaline release. The increased secretion of TSH on exposure to cold depends in turn on the hypothalamus: suitably placed lesions in the hypothalamus can result in diminished thyroid secretion and electrical stimulation can increase it. If the pituitary stalk is cut, there is no thyroid response to cold. The action of thyroid hormone is usually said to be on catabolism while that of noradrenaline is on increasing heat production (Carlson, 1960; Smith, 1963). Fregly, Iampietro and Otis, (1961) confirmed the importance of the thyroid for the survival of rats in severe cold, but showed that cold can induce a rise in metabolic rate in the absence of the thyroid. But thyroidectomized rats lose heat at a greater rate than controls, evidently owing to loss of the ability to constrict peripheral blood vessels.

3.8.6.4. Adrenal Medulla

As mentioned earlier, the occurrence of non-shivering thermogenesis in the cold-acclimated animal has been well demonstrated, at least in the rat (Sellers, Scott and Thomas, 1954; Cottle and Carlson, 1956; Hart, Heroux and Depocas, 1956; Davis, Johnston, Bell and Cremer, 1960). Such an increase

in non-shivering thermogenesis may be due either to greater release of catecholamines or to a greater sensitivity of acclimated animals to these hormones or to both mechanisms. An increased excretion of adrenaline and/or noradrenaline has been reported in rats chronically exposed to cold (Cottle, 1960; Leblanc and Nadeau, 1961). A greater sensitivity of cold-acclimated rats to the calorigenic effect of adrenaline was first observed by Ring (1942) and confirmed later by other workers (Hsieh, Carlson and Gray, 1957; Swanson, 1957). Hsieh and Carlson (1957) also reported a striking calorigenic action of noradrenaline in cold-acclimated rats which was confirmed by Depocas (1960a,b).

Although the calorigenic effects of both adrenaline and noradrenaline are intensified in cold-acclimated rats, acceptance of noradrenaline as the mediator of non-shivering thermogenesis rests on the following observations (Depocas, 1961). Adrenal demedullation lowers the increase in oxygen consumption of the curarized cold-acclimated rat at 6°C (Cottle and Carlson, 1956), but does not abolish it as does previous injection of sympatholytic and ganglion-blocking agents (Hsieh, Carlson and Gray, 1957). Noradrenaline is more effective than adrenaline in preventing the fall in oxygen consumption caused by hexamethonium in curarized cold-acclimated rats (Hsieh, Carlson and Gray, 1957). Cold-acclimated rats show

a greater sensitivity to injected noradrenaline than adrenaline (Hsieh and Carlson, 1957). There is a relationship between the calorigenic response and the dose of noradrenaline administered, a relationship which is dependent on the time of exposure to cold (Depocas, 1960a,b). An inverse relation exists between the increase in the metabolic response to noradrenaline infusion during acclimation to cold (Depocas, 1960a,b) and the decrease in muscle electrical activity (Hart, Heroux and Depocas, 1956) suggesting a substitution of non-shivering thermogenesis under the control of noradrenaline for the shivering heat production. Neither cold exposure nor noradrenaline induce marked hyperglycemia as would be obtained by similar doses of adrenaline (Hsieh and Carlson, 1957).

It can therefore be concluded that non-shivering heat production, associated especially with acclimation to cold, is mediated through the release of noradrenaline, presumably from the adrenergic nerve endings. In this way, the catecholamines play a role in the immediate response to cold exposure.

3.8.6.5. Adrenal Cortex

It is well accepted that the adrenal cortex is specifically involved in the initial response to cold (Sellers, 1957). An increased secretion of corticosteroids has been

demonstrated by many authors in animals suddenly exposed to cold. Adrenalectomized animals have been shown to be unable to survive if left in a cold room (Sellers, You and Thomas, 1951). Whether such an increased secretion persisted in animals maintained in a cold environment for a prolonged period, and whether it was essential for the development or maintenance of cold acclimation, was uncertain until 1951, when Sellers showed that cold acclimated rats after adrenalectomy could be maintained in the cold for long periods by giving a constant but relatively small amount of cortical extract daily. This observation suggested that much less cortical hormone was required for survival after the animals were acclimated to cold than at the beginning of the cold exposure. It was shown by Heroux and Hart in 1954 that eosinophil response to ACTH activity of the adrenal cortex in cold-exposed rats reaches a maximum in about 20 days and then returns to its preacclimation level.

Rossiter and Nicholls in 1957 showed that the increase in the incorporation of inorganic phosphate labeled with radioactive phosphorus into the inorganic phosphorus of the adrenal gland was less in rats acclimated to cold than in rats acclimatized to 22°C when both groups were exposed to -5°C for 2 hours. While this indicates that the adrenal cortex response to a cold temperature shock is less after cold acclimation than before, it did not indicate the state

of activity of the adrenal cortex of the rats kept at their acclimation temperature. In 1960 Heroux measured in vitro the production rate of corticosteroids in adrenals of warm (30°C) and cold (6°C) acclimated rats. The results suggested that an increase in secretion of corticoids is not essential for development of cold resistance as it is possible to develop a certain degree of cold acclimation in adrenalectomized rats provided the animals are exposed to cold gradually and intermittently (Heroux, 1955).

The decreased adrenal response to cold after cold acclimation has been tentatively attributed to an alteration in the sensitivity of the mechanism, nervous or otherwise, by which the pituitary is stimulated, because after cold acclimation the adrenal cortex responds normally to ACTH, vasopressin or adrenaline, indicating clearly that the sensitivity of the hypothalamic-pituitary-adrenal system to a given stimulus is normal (Nicholls, Molloy, Stavraky and Rossiter, 1959). But maintenance of body weight after adrenalectomy with a lower dose of cortical extract in cold than in warm acclimated rats suggested rather a reduced corticosteroid requirement after cold acclimation (Heroux and Hart, 1954).

In agreement with Heroux, in 1960, Schönbaum (1960) found a somewhat reduced adrenal activity in rats exposed

to cold for several months, though there was an initial increase in adrenal activity.

In summary, there seems to be very little doubt now that, while the adrenal cortex is specifically involved in the initial response to cold, it is not essential for maintenance of cold acclimation; it is not even essential for development of a certain degree of cold resistance, provided the animals are exposed to cold gradually and intermittently. Under the fluctuating seasonal conditions prevailing outdoors, however, adrenal cortex appears to remain hyperactive.

Further discussion of the response of the adrenal cortex to cold exposure will be left to a later section.

3.8.6.6. Sex Glands

Repeated observations have shown that in the cold-exposed rat, the testis undergoes some form of degeneration. In a chronic exposure to moderate cold (2°C), this degeneration appears at approximately 40 weeks as a loss of function, exocrine and endocrine, along with morphological modifications, such as changes in weight, alterations of the histological picture of the tubules, etc. (Dugal, Saucier and Des Marais, 1962). In acute exposure to severe cold (-5°C), the same process becomes

observable within a period of 11 days: the testis shows considerable decrease in weight, and some accessories, especially prostate and seminal vesicles, possess the typical eunuchoid charcteristics (Perrault and Dugal, 1963).

One common feature is the position of the testis in the abdominal cavity; indeed, the testes migrate reflexly into the abdomen shortly after the beginning of the cold exposure. The testes remain abdominal as long as the cold stress is applied and at postmortem the animal may be described as cryptorchid.

In summary cold per se produces inhibition of the endocrine function of the testis, demonstrated by a regression of the sexual accessories, prostate and seminal vesicles, to a level approximating that of the castrate.

The cryptorchism produced reflexly by the cold, is not per se responsible for the inhibition of the androgenic function of the testis (Perrault and Dugal, 1962).

Further changes in the function of the sex organs on exposure to cold have been discussed earlier in Section 3.5. on Reproduction.

3.9. HEAT LOSS

3.9.1. INTRODUCTION

It is known that the heat loss of a warm surface to an environment of air is by three main routes, excluding loss by evaporation. These are: convection, radiation, and conduction. Heat loss by convection occurs when air is brought in contact with the surface either by forced or natural currents of air which then take up the temperature of the surface, and carry it to distant points. Heat is lost over the surface of the body because of movement of air (or water) over the surface of the body.

Heat loss by radiation involves an electromagnetic disturbance emitted by the warm surface. This loss would take place even in a vacuum. Air transmits this radiation over long distances but it is eventually absorbed by the molecules of air, so that the air in the environment tends to rise in temperature. Radiant heat loss is thus independent of air movement. In contrast, convective heat loss is very greatly increased by the greater movement of the air, as in a wind.

A third mode of transport of heat into the environment is by conduction, by which means the air in contact with the warm surface is heated, after which the warmed air moves away and cool air takes its place. Obviously, the more air movement, the greater the heat loss by conduction. Thus,

one feels colder in a cold wind than he does in the same temperature when there is no air movement.

Heat flow from an animal to its environment obeys Fourier's law, which states that heat flow between two regions is proportional to the temperature difference and to the thermal conductance (Kleiber, 1961). Kleiber disagrees with the commonly held view that heat loss takes place according to Newton's law of cooling, which states that the rate of cooling is proportional to the temperature difference between the body's surface and the surroundings. He points out that inasmuch as the body's temperature is regulated, a simple law of cooling does not apply. In practice, the difference between these laws is not great; but when, for example, thermal insulation is changing, Fourier's law is superior, since it deals with a regulated flow rather than one which is diminishing as the body cools.

When a mammal is exposed to cold, there are usually changes both in heat production and in heat loss. Sometimes the response is almost wholly either one or the other. The effect on laboratory rats of exposure to cold in the laboratory is a rise in metabolic rate; but wild rats, exposed to the more complex situation of winter out of doors, also improve their insulation (Heroux, 1962). In a discussion on

differences of insulation between individuals, Kleiber (1947) quotes an experiment carried out by Hoesslin in 1888. Hoesslin reared two dogs from the same litter, one at 32°C and the other at 5°C. Kleiber points out that the animal in the cold had to cope with a temperature difference between body and environment six times as great as that to which the other dog was exposed; yet the cold-exposed dog's metabolic rate was only 12% higher. The cold-exposed dog's hair, however, weighed three times that of its litter mate. The acute response to cold must always be primarily metabolic. Even in the acute case, however, an insulative component enters in the form of changes in posture, pilo-erection and, if exposure to thermal neutrality (at which animals produce heat at a minimum level) preceded the cold, peripheral vasoconstriction as well.

Hart (1964a) refers to the presence of thinly furred tissues in the appendages of heavily insulated mammals as solving the problem of exercise heat on the one hand, and conserving heat by cooling on the other. There is considerable variation in skin temperature in the appendages; this is in contrast to the skin under thick fur, which remains uniformly warm. Hart points out further that the cooling of peripheral tissues has an insulating effect. This is accompanied by a remarkable ability to withstand changes of temperature, and by the presence of structures which allow alterations in the

rate of heat loss.

Heat exchange in homeotherms has also been discussed by Scholander (1958). Mammals in the cold are protected from heat loss largely by their fur, yet the legs and other appendages of arctic mammals are poorly insulated, and seals, for example, swim about in very cold water with hairless flippers. If these appendages were kept warm, the heat loss would be very great.

Scholander (1958) discusses the way in which the extremities of cold-adapted mammals are held at a low temperature. Heat from arterial blood is shunted back into the veins before the blood reaches the periphery. The testicle of most mammals is provided with a counter-current vascular system, and this no doubt contributes to keeping it at a temperature lower than that of the body core (Waites and Moule, 1961). Heat loss, rather than conservation, is a main function of the hairless tail and feet of rats (Thompson and Stevenson, 1965). Exposure to acute cold frequently leads to the freezing of the tail which becomes gangrenous and falls off, thus eliminating a source of heat loss. Such tail loss could be regarded as part of the process of protection against the cold.

3.9.2. THE THERMAL INSULATION OF CLOTHING, FUR AND PILO-ERECTION

The recognition of the role of 'dead air' in providing the best thermal insulation obtainable was of tremendous importance in the study and development of thermal insulation in clothing. When it was seen that the insulating properties of a fabric were due, not to the textile fibres directly, but to the dead air entrapped, and more precisely that the thermal insulation of clothing is proportional to the thickness of dead air enclosed, much futile search for better insulation per unit thickness was eliminated.

Homeotherms, other than man, have additional protection in the form of hair or feathers. Furred or feathered animals can maintain body temperature even in subzero weather. They utilize increased heat production by shivering, decreased heat loss by vasoconstriction and the countercurrent systems, and in addition, they have recourse to effective pilo-erection. The term pilo-erection has reference to the elevation of the hair or feathers by the contraction of the muscle attached to the follicle, thus making the hair stand out from the skin rather than lying flat. In effect, pilo-erection increases the depth of the hairy covering. When all the hairs lie flat, the animal wears a thin fur coat; when they stand up straight, the fur coat

is considerably thickened. The thickening keeps the cold air farther away from the skin, and it also decreases the movement of air next to the skin thus decreasing heat loss by convection. The muscles which control pilo-erection are regulated by the sympathetic nervous system as are the muscles which are responsible for vasoconstriction and for shivering. All three, pilo-erection, vasoconstriction, and shivering are regulated automatically by the brain in response to messages from the skin receptors.

3.9.3. VASOCONSTRICTION

The blood whose temperature in man is 37°C, flows to all the vital organs of the body. If the temperature of these organs is to be kept constant, quite obviously, the temperature of the blood flowing through them must be held constant. The problem, then, is to prevent undue heat loss from the blood as it flows through the exposed areas of the skin. Most of the large arteries, which are deep beneath the surface of the body, are insulated by muscle and fat. Blood from the arteries flows through arterioles and then into capillaries. Arterioles, by opening and closing of their diameter, control the blood flow into the capillaries. In fact, blood flow through the skin exposed to very cold air can be decreased to less than 1% of the flow that takes place when the skin is

heated. In cold weather the arterioles through which blood flows to skin capillaries undergo vasoconstriction. The wall of the arterioles contains a thick, circular band of smooth muscle fibres. When the muscle contracts, the diameter of the vessel decreases. These muscles are controlled by the sympathetic nervous system. Thus, the sequence is as follows: the cold air causes the skin receptors to fire, messages are transmitted by sensory nerves to the muscles of the walls of the arterioles, and as a result, they constrict. Blood flow to the skin is therefore impeded.

Vasoconstriction reduces the blood flow to the skin and, in this way, reduces heat loss from the circulating blood. But still there will be some loss, especially from the long extremities, the arms and legs, the fingers and the toes.

3.10. ACCLIMATIZATION TO COLD

The term of acclimatization describes changes in the responses of the organism produced by continued alterations in the environment. Fry, in 1947, has clearly shown that in cold-blooded animals, e.g. fish, acclimatization can take place over a considerable temperature range. In warm-blooded animals considerable adaptation takes place too. A detailed comparison has been made between Arctic and tropical mammals by Scholander, Walters, Hock and Irving in 1950. They observed that birds

walked about at temperatures of -40°C to -50°C without frostbite. But a gull which was kept indoors at 20°C escaped into the snow at -20°C and the web of the feet froze hard within a minute with subsequent gangrene. This might be considered as an example of loss of acclimatization.

Irving in 1951 summarized these studies and concluded that climatic adaptation can be achieved by variation in body temperature, insulation and metabolism. There is no evidence that deep body temperature varies significantly in mammals in different parts of the world. Body temperatures were measured in arctic animals in the winter immediately after they were shot in the bush and averaged 37°C. Reindeer and dogs living at temperatures of -45°C had normal rectal temperatures, so it can be concluded that adaptation to cold is not achieved by a lowering of body temperature. The metabolic cost of living in the Arctic is stated to be about the same as in warmer climates. This statement is based on the findings that the basal metabolic rate in the arctic animals is proportional to size and weight.

Irving concluded that insulation is the most important factor of adaptation to cold in arctic animals.

4. HYPOTHERMIA AND RESUSCITATION

Man and other animals can sustain a considerable fall in body temperature and survive. Hypothermia can be induced by exposure of nude subjects to various environmental temperatures, by immersion in cold water, by packing the subject with ice bags, or by surrounding the subject with rubber tubing through which a refrigerant flows. All these methods have been employed, both experimentally and therapeutically.

There has been extensive animal work on the effects of hypothermia which throws light on the effects of cooling in man. Lutz in 1943 considered that anoxia was a major factor in causing death and claimed good results with oxygen. However, Noell in 1945 examined the EEG during hypothermia and concluded that the changes were not typical of anoxia or anaesthesia, but were similar to eserine poisoning. He suggested that the breakdown of acetylcholine might be delayed at low temperatures.

These workers considered that cardiac failure, which appears to be the main cause of death in hypothermia, was due to the direct effect of cold on the pacemaker of the heart.

The cardiovascular response to hypothermia may be summarized as follows: according to König (1944), there is

an initial marked peripheral vasoconstriction with a rise in blood pressure and heart rate. During this stage the rectal temperature does not fall. If cooling is continued or is intensified, rectal temperature starts to fall. As it does so, the heart rate decreases, and this effect is due to the lowered temperature of the pacemaker. The slowing is not abolished by atropine or vagotomy (Haterius and Maison, 1948; Hegnauer and Penrod, 1950). Blood pressure falls during this period but only gradually. There is an accompanying haemo-concentration due to a fluid shift from the plasma to the tissues. Heart rate becomes progressively slower and arrhythmias appear, and at rectal temperatures of 30°C or lower auricular fibrillation is common. When the rectal temperature reaches a level of 25°C or thereabouts in man and in the dog, blood pressure may fall precipitously and the animal dies owing to ventricular fibrillation. This lethal temperature is variable, to a few degrees in different cases, being 5° to 10°C lower in smaller mammals such as rats and mice.

The proper treatment of hypothermia can be summed up as either slow or rapid re-warming, never moderate re-warming. In acute hypothermia, the main danger is a further fall in body temperature; rapid re-warming is the most effective treatment. In chronic hypothermia body temperature does not fall so fast, but the duration of exposure produces marked

changes in blood volume and depletion of glycogen reserves. Rapid re-warming with intravenous glucose and specific cardiac therapy is one suggested method of treatment. The alternative is a very slow rise in body temperature, possibly combined with glucose. Moderate re-warming has been condemned.

Re-warming of animals cooled to just above and even just below the freezing point has been achieved (Smith, 1961).

5. PROBLEMS AND SUMMARY

Many problems of resistance to cold await elucidation, e.g. genetical problems including the relationship of hybrid vigour with resistance to cold, and the differences between wild and laboratory varieties of the same species.

Little is known about the effects of early exposure to cold on subsequent development. Exposing an adult to cold can improve its resistance to other adverse conditions, but the mechanism of this crossed resistance (and of the opposite, crossed sensitization) is little known.

A complete account of cold-adaptation in mammals requires information on the effects of cold on reproduction, and on the characteristics of the young of cold-exposed females.

Also, the role of early experience other than the exposure to cold and/or learning on the ability to survive the cold awaits clarification.

The many changing processes, reflex, behavioral, enzymic and trophic, involved in adaptation to the cold present problems for which multivariate analyses will undoubtedly be involved. With man exploring the polar regions for vitally needed raw materials and, indeed, with man's exploration of outer space and the pressure of the world's exploding population for places in which to live, more information will be required on man's ability to survive in extremely cold regions on earth and perhaps beyond. This should act as a stimulus for further research in thermoregulatory processes.

PART II

EXPERIMENTAL SECTION

CHAPTER I

EXPERIMENTAL SECTION

THE AIMS OF THE THESIS

The specific aims of the present series of investigations was to elucidate the role of the adrenal cortex in mice exposed intermittently to acute cold (-10°C) and to study the changes in adrenal cortical function during adaptation to this specific stress. To this end, the animals were investigated prior to exposure to cold and during various periods in the cold up to and including animals that became comatose as a result of failure to maintain body temperature. The studies were further continued in the comatose animals after removal from the cold and during the period of recovery. Studies were also conducted in animals exposed to -10°C a second time a week after a similar exposure to -10°C and the pattern of response during the second exposure was compared with that of the first exposure. Both plasma and urinary corticosterone were determined as were also the levels of haemoglobin, blood urea nitrogen and plasma creatinine.

1. INTRODUCTION

During recent decades a great many experiments have been conducted which demonstrated the importance of the adrenal cortex in protecting animals against many different types of stress. Some of the critical early experiments have already been mentioned in the historical section. Studies of changes in adrenal cortical function during exposure to the specific stress of cold have been reported, and they have the following features in common: the exposures were (i) continuous, not intermittent as in this project, (ii) under a relatively mild condition of cold, that is, above 0°C, compared to well below 0°C in the present study, (iii) in rats which because of their larger size tolerate the cold better than mice, (iv) and generally in pooled plasma rather than in samples from individual mice. No studies on the changes in adrenocortical function during and after the intermittent exposure of mice to extreme cold have so far been reported in the literature. Such intermittent exposure tends to mimic more closely the natural conditions to which animals in cold climates are exposed as they move in the search for food from the relative warmth of their sheltered places where they may have nests to the colder temperatures of more exposed areas. Also, the normal day-to-day changes in atmospheric temperatures which occur in

cold climates tend to produce an intermittent stress on the animals. In short, the reports of studies of adrenocortical function under the mild rather artificial conditions of earlier studies indicate that the changes are minimal as will be reported later.

Additional facets of the present project not previously investigated are the changes in adrenocortical function (i) when the animals became comatose when their defences had been overcome by exposure to extreme cold, (ii) on the recovery of such animals from the coma into which the animals had fallen as a result of the exposure to extreme cold, and (iii) during the process of adaptation on repeated exposure to extreme cold.

2. MATERIALS AND METHODS

Animals used in the project were CF_1 female mice 12 to 16 months old, housed in 27-28°C, at 45-55% humidity and illuminated from 7:15 a.m. to 8:30 p.m. EST. The animals were allowed to eat Purina Fox Chow and drink water *ad libitum* before and after exposure to cold. During exposure to -10°C the water bottle was removed from the cage but the food was left in the cage. Animals were removed from the cold room at different time intervals before they

became comatose or when they reached this state, decapitated at room temperature and the blood was collected in heparinized tubes, centrifuged and frozen until assayed. Mice to be decapitated after collapse were housed at 27-28°C until the time selected for sacrificing them. All mice were housed individually at all times during the experiment.

Plasma corticosterone (PC) and haemoglobin (Hb) were determined in each of 82 mice exposed once to -10°C and to their 48 controls, and to 66 mice exposed twice to that temperature. Blood was taken from the mice at various times of the day as required by the schedule shown in the tables containing the data. Determinations of blood urea nitrogen (BUN) and plasma creatinine were carried in the same plasma samples but were pooled from 6 samples to provide the volumes required for the determinations. Urinary corticosterone (UC) was determined in each of 12 mice exposed once and 12 exposed twice to -10°C.

The urine was collected for 24 hours from mice 1 and 7 days after 1 and 2 exposures to -10°C. Collections were made from the mice isolated in metabolic cages containing food and water. Each day the volume of urine collected was measured following which the cage bottom was washed down with distilled water and collected in the same bottle containing the urine. The volume of urine and distilled water was also measured.

Body weights and colonic temperatures were determined at critical times throughout the experiments. The latter variable was determined by means of TRl-R electronic thermometer.

Animals housed in the cold until they became comatose presented the following features: a colonic temperature below 21°C but above 10°C, slowed respiration, heart rate and overall metabolism, flaccidity, and a difficulty of righting themselves when placed on their sides. If animals in this condition were not removed from the cold within 5 to 20 minutes of becoming lethargic, they died. However, if removed to room temperature, that is, around 20°C, about 90% recovered within about an hour.

2.1. METHOD FOR THE DETERMINATION OF THE CORTICOSTEROIDS

The method used in this work for the determination of the corticosteroids have been described earlier, (Murphy, 1967).

2.1.1. THE PRINCIPLE

The principle of the method involves the competitive binding of labelled and unlabelled corticoids by a globulin (CBG) normally found in the serum or plasma which specifically binds the corticosteroids. A solution containing CBG from a standard plasma (in this study dog plasma was utilized)

and labelled corticosterone was added to the unknown and an equilibration immediately occurred between the unbound corticoid and that bound to the CBG. The amount of labelled corticoid now bound to the CBG was inversely proportional to the amount of unlabelled corticoid originally present in the unknown. Florisil was then added which removed the free corticoids, both labelled and unlabelled, and the amount of labelled corticoid bound to the CBG was estimated by an appropriate radioactive-detecting device (scintillation spectrometer). The amount of corticoid originally present in the unknown was read off from a standard curve which had a range of from 0 to 8 ng. Values have been obtained in plasma samples as low as 5 lambda, but usually in 10 or 20 lambda, and in urine volumes of 100 lambda. The small volumes and short time required for the assay are the main advantages of this method. Tests of specificity, precision and accuracy have been reported earlier (Murphy, 1967; Grad and Khalid, 1968).

2.1.2. THE PREPARATION OF THE CBG-ISOTOPE SOLUTION (SOLUTION A)

Into a 100 ml volumetric flask containing 15-20 ml of distilled water, add 2.5 ml dog's plasma or serum. Then add 0.4 ml of a solution of corticosterone-H^3 (10 μc/ml) in ethanol (specific activity 158 μc/μg). Make up to a final volume of 100 ml with distilled water.

2.1.3. THE PREPARATION OF THE STANDARD CURVE

2.1.3.1. PC and UCs Standards

From a corticosterone solution of 100 mμg/ml ethanol, pipette in triplicate 0, 0.01, 0.02, 0.03, 0.04, 0.08 ml to give a 0, 1, 2, 3, 4, 8 mμg standards. Evaporate under a gentle stream of filtered air.

2.1.4. THE PREPARATION OF THE UNKNOWNS

2.1.4.1. Plasma Samples

Pipette 0.01 ml plasma into centrifuge tubes, add 1 ml ethanol in duplicate, mix and centrifuge for 4 minutes. Decant supernatant carefully into test tube. Evaporate for dryness. The starting plasma volume utilized in this study was varied from 0.005 to 0.05 ml depending on the previously estimated PC level.

2.1.4.2. Urine Samples

Into a centrifuge tube, pipette 0.1 ml urine in duplicate, add 1 ml methylene chloride, mix and centrifuge for 5 minutes. Transfer the extract into a test tube and repeat extraction with another 1 ml of methylene chloride. Evaporate to dryness.

2.1.5. PREPARATION OF COUNTING SOLUTION

The counting solution used was Bray's solution, prepared as follows: to one U.S. gallon of dioxane, 400 gm. naphtalene 28 gm. PPO (2,5 - diphenyloxazole) and 1.2 gm. dimethyl POPOP (4-methyl-5-phenyloxazolyl-benzene) were added to the bottle containing the dioxane. All were shaken together and allowed to stand overnight. The bottle was not filled to the top as its contents expanded overnight.

2.1.6. PREPARATION OF STANDARD CHECK

To check on the amount of radioactivity of the CBG-isotope solution, 1 ml is added to 10 ml of Bray's solution and counts are recorded. This was done both for the PC and UC determinations. Ansitron liquid-scintillation spectrometer with a counting efficiency of 10.5% for tritium was used for counting the urine samples, and Packard liquid-scintillation with 33% efficiency for counting the plasma samples.

2.1.7. DETERMINATION OF STANDARDS AND UNKNOWNS

Into each test tube containing the dried solutions of standards and unknowns, add 1 ml of CBG-isotope solution (solution A). Mix well. Warm to 45°C for 5 minutes. Cool to 10°C for 20 minutes. To each test tube, add 40 mg Florisil (measured with a specially designed plastic spoon).

Shake for 2 minutes on an automatic shaker, then return to the 10°C bath for 10 minutes. Florisil settles to the bottom. Pipette 0.5 ml supernatant into 10 ml Bray's solution and shake well. Count each sample to 5000 twice in a liquid scintillation counter. Plot the time required to count the standards as ordinates versus the concentration (mμg) of the unlabelled steroids as abscissae. This yields a standard curve off which the unknowns are read.

2.1.8. CALCULATIONS

PC: the value read off from the curve x 10 (for 0.01 ml plasma) = μg/100 ml.

UCs: The value read off from the curve ÷ 0.1 (for 0.1 ml urine) x the total volume of urine collected in 24 hours = ng/24 hours.

2.2. THE DETERMINATION OF Hb, BUN AND PLASMA CREATININE

The Hb determinations were conducted according to Davidsohn and Wells (1963) while BUN and plasma creatinine were determined as described by Reiner (1953).

2.3. STATISTICAL METHODS

Means and standard errors were calculated for the data. Several types of statistical analysis were utilized:

one-way analysis of variance and two types of two-way analysis of variance, one in which each factor was independent (Snedecor, 1966) and the other in which one of the factors was dependent, that is, there were repeated measures in one of the factors (Winer, 1962); t tests were calculated where applicable. Correlation coefficients between the variables were also calculated. A probability less than 0.05 was considered significant.

3. RESULTS

3.1. THE EFFECT OF EXPOSURE TO -10°C ON THE PLASMA CORTICOSTERONE LEVEL, COLONIC TEMPERATURE AND BODY WEIGHT OF CF_1 FEMALE MICE

3.1.1. THE EFFECT OF A SINGLE EXPOSURE TO -10°C (TABLES 1,2 and 3)

Table 1 presents the PC and colonic temperature (CT) data of mice housed and adapted to 27°C and sacrificed at various times during the day. The values varied from 4.2 ± 0.4 mcg% at around 11 a.m. to a high of 13.0 ± 1.5 mcg% between 5 and 6 p.m., a highly significant difference ($P < 0.001$). The non-cold-exposed mice were sacrificed at the same times of the day as those exposed to the cold. This was to make it possible to separate the influence of diurnal variation on the PC level from the effect of exposure to -10°C.

The PC level of mice exposed once to -10°C for various times and other relevant data are presented in Table 2.

Table 1. The Colonic Temperatures and Plasma Corticosterone Levels of CF_1 Female Mice Exposed Once to -10°C For Various Times and of Non-Exposed Control Mice Sacrificed at The Same Time

Time at -10°C	Time Mice Sacrificed After Cold Exposure*	Time of Decapitation	Non-Exposed		Exposed to -10°C	
			Colonic Temperature at Decapitation (C)	Plasma Corticosterone (µg ·/.)	Colonic Temperature at Decapitation (C)	Plasma Corticosterone (µg ·/.)
60 min.	0	10:15 ± 6	36.4 ± 0.3	5.6 ± 1.5	32.4 ± 1.2	56.0 ± 3.9
Until Comatose	0	10:58 ± 2	36.7 ± 0.6	4.2 ± 0.4	18.1 ± 0.6	41.7 ± 3.9
" "	60 min.	12:00 ± 9	36.6 ± 0.3	6.0 ± 1.7	34.7 ± 0.8	50.5 ± 3.5
" "	120 min.	14:30 ± 15	36.6 ± 0.2	6.0 ± 1.7	35.9 ± 0.5	19.3 ± 3.3
" "	240 min.	15:05 ± 5	36.6 ± 0.3	8.9 ± 1.7	35.6 ± 0.8	22.5 ± 3.7
" "	360 min.	17:40 ± 10	36.1 ± 0.3	13.0 ± 1.5	35.6 ± 0.4	26.5 ± 7.0
" "	1 day	11:40 ± 20	37.3 ± 0.3	9.2 ± 1.7	37.4 ± 0.2	8.9 ± 4.1
" "	14 days	11:40 ± 20	37.0 ± 0.2	7.9 ± 2.3	36.4 ± 0.4	15.2 ± 3.5

Footnote on page 102.

Table 1.

The above means and standard errors were calculated for 6 animals per group except in the case of "Comatose + 7 days" group where 4 mice were studied.

*Mice investigated at various time intervals after becoming comatose at -10°C were housed in the warm room at 27-28°C until sacrificed.

Table 2. The Colonic Temperatures And Plasma Corticosterone Levels of CF_1 Female Mice Exposed Once to -10°C For Various Times And Sacrificed At Various Times Thereafter

Time at -10°C	Time Mice Sacrificed After Cold Exposure*	Colonic Temperature (°C) Pre Exposure	Immediately Post Exposure	at Decapitation	Plasma Corticosterone (µg ·/.)
5 min.	0	37.5 ± 0.2	36.6 ± 0.2	36.6 ± 0.2	19.0 ± 1.9
15 "	0	36.8 ± 0.3	34.6 ± 0.8	34.6 ± 0.8	43.5 ± 2.1
60 "	0	36.6 ± 0.6	32.4 ± 1.2	32.4 ± 1.2	56.0 ± 3.9
Until Comatose	0	38.0 ± 0.2	18.1 ± 0.6	18.1 ± 0.6	41.7 ± 3.9
" "	30 min.	38.2 ± 0.5	13.8 ± 0.6	20.7 ± 0.3	36.7 ± 3.9
" "	60 "	37.3 ± 0.4	18.9 ± 0.4	34.7 ± 0.8	50.5 ± 3.5
" "	120 "	37.7 ± 0.3	15.1 ± 0.4	35.9 ± 0.5	19.3 ± 3.3
" "	240 "	37.0 ± 0.3	14.9 ± 0.2	35.6 ± 0.8	22.5 ± 3.7
" "	360 "	37.0 ± 0.3	15.0 ± 0.5	35.6 ± 0.4	26.5 ± 7.0
" "	1 day	36.8 ± 0.4	15.0 ± 0.6	37.4 ± 0.2	8.9 ± 4.1
" "	2 "	37.0 ± 0.2	15.8 ± 0.8	37.7 ± 0.2	3.3 ± 0.7
" "	4 "	37.3 ± 0.3	15.7 ± 0.4	37.0 ± 0.6	13.8 ± 2.8
" "	7 "	37.2 ± 0.2	15.3 ± 0.7	36.8 ± 0.5	5.9 ± 0.9
" "	14 "	36.7 ± 0.3	14.9 ± 0.5	36.4 ± 0.4	15.2 ± 3.5

Footnote on page 104.

Table 2.

The above means and standard errors were calculated for 6 animals per group except in the case of "Comatose + 7 days" group where 4 mice were studied.

*Mice investigated at various time intervals after becoming comatose at -10°C were housed in the warm room at 27-28°C until sacrificed.

Table 3. Analysis of Variance of Plasma Corticosterone Level (mcg%) in Cold Exposed Mice of Table 1

Source of Variation	Degrees of Freedom	Mean Square
Exposure (E)	1	12069.14[a]
Time of Exposure (T)	7	772.54[a]
E x T	7	1669.51[a]
Error	80	8.50

[a]Significant at the 0.05% level

An analysis of variance of the PC data showed a significant difference between the various groups ($P < 0.0005$). A 5 minute exposure to -10°C resulted in a PC level of 19.0 ± 1.9 mcg% (Table 2). This was significantly above the control level at 5.6 ± 1.5 mcg% shown in Table 2 ($P< 0.001$). An exposure of 15 minutes in the cold resulted in a PC level of 43.5 ± 2.1 mcg%, a further significant increase ($P < 0.001$), and still another significant increase occurred in mice exposed to -10°C for 60 minutes, 56.0 ± 3.9 mcg% ($0.05 > P > 0.02$). Mice left in the cold until they became comatose had a PC level of 41.7 ± 3.9 mcg%, a significant fall compared to that of mice exposed to the cold for an hour ($0.02 > P > 0.01$).

Blood collected from mice that had been rendered comatose at -10°C and then returned to the warmth for 30 minutes showed no significant change from the previous reading (Table 2, 36.7 ± 3.9 mcg%, $0.50 > P > 0.40$). Mice housed in the warmth for an hour after becoming comatose at -10°C and then sacrificed showed a further increase in PC level to 50.5 ± 3.5 mcg%, a significant increase compared to the previous value ($0.05 > P > 0.02$). Mice sacrificed 2 hours after becoming comatose at -10°C showed a significant fall in PC level to 19.3 ± 3.3 mcg% ($P < 0.001$), and animals similarly treated but sacrificed during the next 4 hours had PC values which remained more or less at the same level. However, animals sacrificed a

day after developing coma at -10°C showed a significant drop in PC level to 8.9 mcg% ($0.01 > P > 0.0001$), and a day later the values fell to 3.3 ± 0.7 mcg% the lowest values in the entire series but still not reliably below those obtained a day before ($0.30 > P > 0.20$). The next two weeks saw the values rise and fall although the differences were not significantly different from those immediately preceeding or following ($P > 0.05$). A two-way analysis of variance with interaction of the PC data of control and cold-stressed animals of Table 1 revealed that the values of the exposed mice were very significantly higher than those of the non-exposed mice (Table 3, $P < 0.0005$). Moreover, the pattern of change of the PC level with time was significantly different in the control and cold-treated mice (Table 3, E x T interaction, $P < 0.0005$). Thus, the peak in the control mice occurred at about 5:40 p.m. while in the exposed mice it occurred at the earlier time intervals.

The CT's of both non-stressed and cold-stressed animals were included in Tables 1 and 2. The means and standard errors of the pre-exposure CT varied from 36.6 ± 0.2°C in one group to 38.2 ± 0.2°C in another. The difference was small when compared to the decline in temperature which occurred in mice exposed to the cold. Thus, the CT of mice exposed to -10°C fell steadily until they became comatose when the CT's were below 20°C, a highly significant fall ($P < 0.001$).

The temperature at the time of decapitation varied from a high of 37.7 ± 0.2°C in animals sacrificed two days after recovering from coma at -10°C to a low of 18.1 ± 0.6°C in comatose mice, a highly significant difference ($P < 0.001$).

All mice lost weight on exposure to the cold, which would be expected in view of the increase in metabolic rate, a phenomena observed earlier (Grad, Levine and Berenson, 1968).

3.1.2. THE EFFECT OF TWO EXPOSURES TO -10°C (TABLE 4)

An analysis of variance of the PC data of mice exposed to the cold twice with a 7-day interval at 26 - 27°C showed that significant differences occurred at the various time intervals ($P < 0.0005$).

Examination of Table 4 shows that the PC levels of mice exposed to -10°C twice for 15 or 60 min. or to coma the first time and for 15 or 60 min. on the second exposure have very significantly higher values than those of the non-stressed mice shown in Table 1 ($P < 0.001$). Of these 4 groups, the mice exposed for the longer periods generally had the higher PC levels. Animals taken to coma at -10°C for a second time, following a similar first experience, also showed a significant fall in the PC level as occurred following the first exposure ($P < 0.001$). On recovery in the warmth, the values remained at the same level for the first hour ($0.40 > P > 0.30$), but declined significantly during the next hour ($0.01 > P > 0.001$), remained

Table 4. The Body Weights, Colonic Temperatures, and Plasma Corticosterone of CF_1 Female Mice Exposed to -10°C 7 Days Apart For Various Times and Sacrificed At Various Times Thereafter

Time Exposed to -10°C First Exposure	Second Exposure	Time Mice Sacrificed After Cold Exposure*	Body Weight at Decapitation (g)	Colonic Temperature at Decapitation (C)	Plasma Corticosterone (ug %)
15 min.	15 min.	0	38.2 ± 0.8	34.8 ± 0.5	37.0 ± 1.4
60 min.	60 min.	0	37.2 ± 1.2	31.1 ± 1.2	51.3 ± 3.0
Until Comatose	15 min.	0	38.0 ± 1.8	35.1 ± 1.1	40.8 ± 1.6
" "	60 min.	0	36.4 ± 1.7	34.4 ± 0.8	48.0 ± 4.0
" "	Until Comatose	0	38.7 ± 0.9	14.2 ± 0.3	26.3 ± 2.4
" "	" "	60 min.	39.0 ± 1.3	24.2 ± 0.9	30.5 ± 4.5
" "	" "	120 min.	38.0 ± 2.1	36.2 ± 0.3	15.8 ± 3.4
" "	" "	360 min.	37.5 ± 1.0	37.0 ± 0.2	19.3 ± 5.4
" "	" "	1 day	36.2 ± 2.2	37.2 ± 0.2	20.8 ± 3.0
" "	" "	2 days	39.6 ± 2.0	37.1 ± 2.0	8.3 ± 1.0
" "	" "	7 days	37.2 ± 1.9	36.5 ± 0.2	6.3 ± 1.3

Table 4.

The above means and standard errors were calculated for 6 animals per group.

*Mice investigated at various time intervals after becoming comatose at -10°C were housed in the warm room at 27-28°C until sacrificed.

steady at the new level until the second day of recovery when a further significant drop occurred ($0.02 > P > 0.01$), at which level it was also found to be on day 7 ($0.70 > P > 0.60$).

Mice exposed to -10°C twice for 15 min. within 7 days showed a significantly lower PC level than those exposed to -10°C for 15 min. only once (Tables 2 & 4, $0.05 > P > 0.02$). Mice similarly exposed twice to -10°C for 60 min. also had a lower PC level than those exposed to -10°C for 1 hour only once but the difference was not statistically significant ($0.40 > P > 0.30$). Furthermore, mice exposed to -10°C the first time until comatose and a week later for 15 min. had PC values which were not significantly different from those with the same 15-min. second exposure but only at a 15-min. exposure the first time ($0.30 > P > 0.20$). The same was true when the 15-min. exposures were extended to 60 minutes ($0.30 > P > 0.20$).

A comparison by analysis of variance of the PC levels of mice exposed to the cold once with those of mice exposed twice revealed that the latter were significantly lower than those of the former (Tables 2, 4 and 5, $0.01 > P > 0.005$). Also, the values of the different times of exposure to the cold were statistically significant , as has already been described (Table 5, $P < 0.0005$). Moreover, the interaction between the latter two factors was highly significant (Table 5, $P < 0.0005$), with the

Table 5. Analysis of Variance of Plasma Corticosterone Levels (mcg%) of Tables 1 and 4 Taken From the "Comatose" to the "Comatose + 4 Days" Groups Inclusive

Source of Variation	Degrees of Freedom	Mean Square
Exposure (E)	1	787.38[a]
Time of Exposure	5	1298.89[b]
E x T	5	659.95[b]
Error	60	95.68

[a]Significant at the 1% level

[b]Significant at the 0.5% level

pattern of PC change following coma in the "one-exposure" mice showing a more marked oscillation on recovery than the "two-exposure" mice. Moreover, between 6 hrs. and 1 day following coma, there was a significant drop in the PC levels in mice having only one exposure (Table 2, $0.01 > P > 0.001$), whereas there was no significant change in those that had two exposures (Table 4, $0.80 > P > 0.70$).

The CT of mice exposed twice to - 10°C tended to decline up to the state of collapse, the pattern of response being similar to that seen earlier in mice exposed to -10°C once (Tables 1 & 2). However, the time to fall into coma a second time was significantly longer than that of the first (110 ± 5.8 min. vs 126 ± 4.8 min., $0.05 > P > 0.02$), a finding reported earlier (Grad et al, 1968).

3.2. THE EFFECT OF ONE OR TWO EXPOSURES TO -10°C ON THE URINARY CORTICOSTEROIDS OF CF_1 FEMALE MICE (TABLES 6 AND 7)

The UC amounts of mice, one day following one exposure, was below that of controls but the difference was not statistically significant ($0.60 > P > 0.50$). However, the UC of mice, one day after two exposures, was significantly below that of controls ($0.05 > P > 0.02$).

When a similar comparison was made by analysis of

Table 6. The Body Weights, Colonic Temperatures and Urinary Corticosteroids Amounts of CF_1 Female Mice Exposed Once or Twice to -10°C Until Comatose and Their Non-Exposed Controls

Number of Exposures to -10°C	Day of Urine Collection After Last Exposure to -10°C	Body Weight (g)		Colonic Temperature (°C)		Urinary Corticosteroids (ng/24 hours)
		Before*	After*	Before*	After*	
0	0	38.7 ± 0.9	38.8 ± 1.0	37.0 ± 0.2	37.1 ± 0.3	340 ± 72
1	1	39.0 ± 0.9	38.9 ± 0.9	36.8 ± 0.1	36.8 ± 0.1	293 ± 24
1	7	38.8 ± 0.8	38.8 ± 0.8	36.9 ± 0.2	36.9 ± 0.2	360 ± 52
2	1	38.2 ± 1.3	38.1 ± 1.2	36.9 ± 0.2	37.0 ± 0.2	174 ± 29
2	7	39.7 ± 0.8	39.7 ± 0.7	36.9 ± 0.2	37.1 ± 0.2	207 ± 46

The above means and standard errors were calculated for 6 animals per group.

*Body weights and colonic temperatures were taken immediately before placing the mice in the metabolic cages for urine collection and immediately after their removal.

Table 7. Analysis of Variance of Urinary Corticosteroid (ng/24 hours) of Mice Exposed to -10°C

Source of Variation	Degrees of Freedom	Mean Square
Between Subjects	11	
A (Number of Exposures)	1	110,048.96[a]
Subjects within Groups	10	6,828.45
Within Subjects	12	
B (Day of Collection)	1	15,050.04[b]
AB	1	2,542.08[b]
B x Subjects within Groups	10	12,316.27

[a]Significant at the 0.5% level

[b]Not significant

variance between the UC of control mice and those of day 7 after the first and second exposures, the differences were not found to be statistically significant ($0.20 > P > 0.10$), although the general trend was the same.

Analysis of variance of the data of mice exposed one and 7 days after one or two exposures revealed that the UC values were significantly lower in mice that had been exposed twice than that of those exposed only once (Table 7, $0.005 > P > 0.001$). Although the values obtained on the seventh day were higher than those obtained on the first day, the differences were not statistically different (Table 7, $0.30 > P > 0.20$); nor was the interaction between the number of exposures and the times of collection of the urine statistically significant (Table 7, $P > 0.50$).

There was no change in body weight or colonic temperature of the animals as a result of one day in the metabolic cages.

3.3. THE EFFECT OF ONE OR TWO EXPOSURES TO -10°C ON THE HAEMOGLOBIN (Hb) LEVEL OF CF_1 FEMALE MICE (TABLES 8, 9 & 10)

The Hb levels determined at each of the time intervals shown in Table 5 varied from a low of 12.4 ± 0.4 g%

Table 8. Haemoglobin, Blood Urea Nitrogen and Plasma Creatinine of CF_1 Female Mice Exposed Once to -10°C for Various Times and Their Non-Exposed Controls Sacrificed Simultaneously

Time at -10°C	Time Mice Sacrificed After Cold Exposure*	Haemoglobin (g%)	Blood Urea Nitrogen (mg%)	Plasma Creatinine (mg%)
0 min.	0 min.	13.2 ± 0.2	15.7	0.61
5 min.	0 min.	12.7 ± 0.4	22.0	0.77
15 min.	0 min.	13.3 ± 0.3	30.7	0.60
60 min.	0 min.	12.9 ± 0.5	36.0	0.57
Until Comatose	0 min.	12.4 ± 0.4	22.7	0.63
" "	30 min.	12.9 ± 0.4	29.0	0.70
" "	60 min.	12.5 ± 0.5	30.7, (22.7)	1.10, (0.44)
" "	120 min.	12.5 ± 0.3	38.0, (19.3)	0.64, (0.56)
" "	240 min.	12.6 ± 0.5	48.7, (18.0)	0.88, (0.56)
" "	360 min.	12.9 ± 0	33.3, (19.3)	0.57, (0.56)
" "	1 day	12.6 ± 0.6	28.0, (24.0)	0.63, (0.44)
" "	2 days	13.0 ± 0.4	21.3	0.64
" "	4 days	13.5 ± 0.4	20.0	0.51
" "	7 days	13.9 ± 0.2	16.6	0.63
" "	14 days	13.0 ± 0.4	-	-

Table 8.

The haemoglobin data are the means and standard errors of 6 mice per group except in the case of "Comatose + 7 days" group where 4 mice were studied. The remaining data are single values obtained from plasma pooled from the same groups of animals. The values in the brackets are those of mice not exposed to -10°C but sacrificed at the same time.

*Mice investigated at various time intervals after becoming comatose at -10°C were housed in the warm room at 27-28°C until sacrificed.

when the animals were comatose to a high of 13.9 ± 0.2 g% when the animals were sacrificed 7 days after they became comatose. However, an analysis of variance carried out over all the data failed to show a significant difference ($P > 0.50$).

An examination of the Hb data of twice-stressed mice (Table 9) shows that there is a tendency for a slight rise to occur when the animals were placed in the cold, that a drop occurs when the animals are in coma, and that the levels increase again as the animals recovered from the coma after being removed from the cold. An analysis of variance showed that the overall difference between the various groups was statistically significant ($0.01 > P > 0.005$). A significant decline occurred in the animals sacrificed immediately after becoming comatose during the second exposure of -10°C (Table 9, 12.0 ± 0.6 g%). At this time, the values were the lowest of all the values in the series and significantly lower than the data of mice in adjacent groups in the table ($0.01 > P > 0.001$).

Comparisons were made by analysis of variance between the Hb data of Table 9 with comparable "one-exposure" groups of 6 mice per group in Table 8. The results showed that the animals exposed twice had significantly higher Hb levels than those exposed only once ($0.025 > P > 0.01$). There was a signi-

Table 9. Haemoglobin, Blood Urea Nitrogen and Plasma Creatinine of CF_1 Female Mice Exposed to -10°C for Various Times and Sacrificed Either Immediately or After Being Housed at 27-28°C for Various Times

Time Exposed to -10°C First Exposure	Second Exposure	Time Mice Sacrificed After Cold Exposure*	Haemoglobin (g%)	Blood Urea Nitrogen (mg%)	Plasma Creatinine (mg%)
15 min.	15 min.	0	13.7 ± 0.2	33.3	0.54
60 min.	60 min.	0	14.0 ± 0.3	33.3	0.51
Until Comatose	15 min.	0	13.7 ± 0.8	29.6	1.00
" "	60 min.	0	14.4 ± 0.4	29.6	0.86
" "	Until Comatose	0	12.0 ± 0.6	29.0	0.57
" "	" "	60 min.	14.2 ± 0.2	34.8	0.91
" "	" "	120 min.	13.4 ± 0.4	38.7	0.91
" "	" "	360 min.	-	29.0	0.86
" "	" "	1 day	12.7 ± 0.4	20.0	1.14
" "	" "	2 days	13.8 ± 0.4	23.8	0.86
" "	" "	7 days	14.5 ± 0.5	21.2	0.86

Table 9.

The haemoglobin data are means and standard errors calculated for 6 animals per group. The remaining data were obtained from plasmas pooled from 6 animals.

*Mice investigated at various time intervals after becoming comatose at -10°C were housed in the warm room at 27-28°C until sacrificed.

Table 10. Analysis of Variance of the Haemoglobin Levels (g%) of Equal Sized Groups of Mice Exposed Once (Table 8) or Twice (Table 9) to -10°C and Sacrificed at the Same Times Following Recovery From Coma on Exposure to Cold

Source of Variation	Degrees of Freedom	Mean Square
Exposure (E)	1	6.21[a]
Time of Exposure (T)	5	3.55[b]
E x T	5	1.52[c]
Error	60	

[a]Significant at the 2.5% level

[b]Significant at the 1% level

[c]Not significant

ficant difference in the time factor with the lowest values occurring when the animals were comatose (Tables 8, 9 and 10, $0.01 > P > 0.005$). This was previously reported for the "two-exposure" mice alone (Table 9), but it was also apparent in the "one-exposure" animals, in which the change was not significant. The pattern of change of the Hb level in the one and two-exposure mice was essentially the same (Tables 8, 9 and 10, $0.20 > P > 0.10$).

3.4. THE EFFECT OF ONE OR TWO EXPOSURES TO -10°C ON THE BUN LEVEL OF CF_1 FEMALE MICE (TABLES 8 & 9)

The BUN values increased immediately after exposure to cold from 15.7 to 22.0 mg% and continued to do so until 36.0 mg% after 1 hour in the cold. The BUN levels remained elevated even when the animals had become comatose, though they had fallen to 22.7 mg% from the high value at 1 hour in the cold. Indeed, the maximum values were achieved only 4 hours after the animals had recovered from the coma following which the values tended to decline and returned to normal only 7 days after the exposure.

The BUN of mice exposed to the cold twice showed elevated levels when compared with that of the control value of 15.7 mg% shown in Table 8. Indeed, the values tended to stay up even when the animals had become comatose and up to 6 hours upon

recovery from coma due to cold. Only the day following the second exposure did the values return to the normal level. There was no significant difference between the BUN of once or twice-exposed mice ($0.80 > P > 0.70$).

3.5. THE EFFECT OF ONE OR TWO EXPOSURES TO -10°C ON THE PLASMA CREATININE LEVEL OF CF_1 FEMALE MICE (TABLES 8 & 9)

The increase in the plasma creatinine which was apparent 5 min. after exposure to -10°C disappeared when the animals had been in the cold for 15 and 60 min. and, indeed, was at the normal level when the animals were comatose. However, marked increases were apparent soon after the animals were brought out of the cold after being in coma and the maximum of 1.10 mg% occurred 1 hour after being removed from the cold room. Thereafter, the values tended to drift downwards and were back at the normal level two days after being exposed to the cold and continued at this level for the next 5 days.

Mice that received two exposures to the cold of 15 min. or 1 hour showed no remarkable change in the plasma creatinine. However, mice that had a first exposure until they became comatose showed elevated levels on second exposure even for as little as 15 min. and these values tended to stay elevated for as long as 7 days following recovery from the second exposure to the cold. In

general, the plasma creatinine values of mice exposed twice to the cold had higher values even than those exposed only once, the difference being of borderline significance ($0.10 < P > 0.05$).

3.6. CORRELATION COEFFICIENTS BETWEEN THE VARIOUS PARAMETERS

There was no significant correlation between the PC level of mice not exposed to -10°C and those exposed to -10°C and sacrificed at the same time ($r = +0.1750$, $P > 0.10$). Similarly, there was no significant correlation between the Hb level on the one hand and the PC level on the other in mice exposed once or twice to the cold (Tables 1, 4, 8, 9, $P > 0.10$).

In mice exposed once to the cold, there was a significant correlation between the mean PC levels (Table 2) and the BUN (Table 8, $r = +0.9039$, $P < 0.001$), but there was no significant correlation between the same PC values and the plasma creatinine (Table 8, $r = +0.2912$, $P > 0.10$). In mice exposed twice to -10°C, there was no significant correlation between the mean PC levels (Table 4) and either the BUN (Table 9, $r = +0.4606$, $P > 0.10$) or the plasma creatinine (Table 9, $r = -0.3616$, $P > 0.10$).

In mice exposed once to -10°C, there was a significant negative correlation between the mean Hb and plasma creatinine levels (Table 8, $r = -0.5556$, $0.05 > P > 0.02$) and also a significant positive correlation between the BUN and the

plasma creatinine level (Table 8, $r = +0.6157$, $0.05 > P > 0.02$). However, there was no significant correlation between the mean Hb and the BUN (Table 8, $r = +0.3109$, $P > 0.10$).

There was no significant correlation between the Hb, BUN or plasma creatinine levels in mice exposed twice to the cold (Table 9, $P > 0.10$).

A significant correlation between body weight and Hb was found to occur in mice exposed once to the cold ($r = -0.3047$, $0.75 > P > 0.02$) but not in those exposed twice (Table 4, $r = -0.3748$, $P > 0.10$).

4. DISCUSSION

4.1. THE METHOD

The index of precision (λ) of the standard curves of this assay were previously calculated by Grad and Khalid (1968) and yielded means and standard errors of 0.195 ± 0.0022. More recently, Grad, Sancho and Murphy (1969) found λ's of 0.110 ± 0.007. These values are satisfacotry and well below the 0.300 level accepted with assays much less sensitive than the one utilized in the present study (Dorfman, 1950).

The precision of the determination of the PC levels in test samples was assessed by calculating the standard deviation of duplicate determinations in C57 and AKR mice (Grad and Khalid, 1968) and more recently in C3H mice (Grad, Sancho and Murphy, 1969). These values were also found to yield satisfactory results.

The precision of the determination of UC in unknowns from mice utilizing the ultramicro assay was found by Grad, Sancho and Murphy (1969) to be as good as that reported earlier for semimicro assays of the UC in humans (Grad, Kral, Payne and Berenson, 1967).

The accuracy of the assay for the determination of the PC levels and UC amounts was assessed previously by determining the percent recovery of one nanogram of corticosterone added to the serum or plasma and urine of C57 and AKR mice (Grad and Khalid, 1968) and to the plasma of C3H mice (Grad, Sancho and Murphy, 1969). The percent recovery was satisfactory in both these studies and was found to be at least as good as the recovery of 100 to 300 nanograms added to pooled urine utilizing a semimicro form of this assay (Grad et al., 1967).

Tests for specificity of the assay were conducted earlier by Murphy (1967) and by Grad and Khalid (1968). Murphy

found that the method involving dog's plasma as the source of CBG could be utilized to determine cortisol or corticosterone. Inasmuch as there is no cortisol or indeed, any 17-hydroxylated steroids in mouse plasma (Triller and Birmingham, 1965), the method is specific for the determination of corticosterone in this biological liquid and probably also in mouse urine.

Thus, the method utilized in the present study was found to be satisfactory for the determination of PC and UC as assessed by standard tests of precision, accuracy and specificity.

4.2. THE CONTROL PC AND UC VALUES OF CF_1 FEMALE MICE

The late morning PC level of CF_1 female mice were 6.8 ± 1.1 mcg% (Table 1). These values are in good agreement with the mean and standard error of 6.3 ± 0.9 mcg% found in female C57Bl/6J mice, 12 to 13 months old, (Grad and Khalid, 1968) and with 8.6 ± 1.6 mcg% in healthy AKR mice, 12 months old (Grad and Khalid, 1969a). However, CF_1 female mice had values which were significantly below the 13.8 ± 2.1 mcg% of C3H females, 9 to 15 months old, ($0.01 > P > 0.001$). Earlier still, Levine and Treiman (1964) reported values between 8 and 9 mcg% in AKR mice while Solem (1966) reported levels of 12.1 ± 1.1 mcg% for WLO female mice. However, these values were for 2 to 3 months old animals and

were obtained by fluorometric techniques which are known to yield higher values due to the presence of a contaminating nonspecific substance.

It is not possible to make accurate comparison with earlier reports from other laboratories because not only are such reports few, but where they are given they are for plasmas that have been pooled from several animals whose age and sex are not given. These parameters have been shown to reliably influence the PC values, females having significantly higher values than males in some mouse strains (Grad and Khalid, 1968) but not in others (Grad, Sancho and Murphy, 1969). There are also reliable changes in PC level with age, some occurring early in life between 2 and 4 months of age and others occurring from maturity to old age (Grad and Khalid, 1968, 1969a).

Table 1 showed that the PC level of the non-exposed mice increased to a maximum of 13.0 ± 1.5 mcg% at around 5:40 p.m., some of the values earlier in the day being reliably lower. This is in agreement with reports published earlier utilizing a less specific method (Halberg, Peterson and Silver, 1959). Because of this significant diurnal variation, it was necessary in the study of the PC levels to sacrifice non-cold-stressed animals simultaneously with animals exposed to the stress, especially as the latter

were being sacrificed at times of the day when it was known that the normal levels were elevated. This aspect of the problem will be further discussed later.

The mean and standard error of UC levels of CF_1 female mice not exposed to the cold were reported as being 340 ± 72 ng/24 hours (Table 6), which are higher than the 112 ± 32 ng/24 hours of 7 to 12 month old female C57 mice (Grad and Khalid, 1969b), higher also than the 117 ± 21 ng/24 hours of 7 to 12 month old healthy female AKR mice, (Grad and Khalid, 1969b) and higher also than the 173 ± 23 ng/24 hours of healthy female C3H mice, (Grad, Sancho and Murphy, 1969). The reason for the higher UC amounts in the CF_1 mice is not known, but CF_1 animals are larger and more vigorous animals and are not inbred whereas the other 3 strains are.

4.3. THE CHANGE IN PC LEVELS OF CF_1 FEMALE MICE ON EXPOSURE TO -10°C

The PC level promptly rose on exposure to -10°C and continued to do so until they became comatose when the values declined, but still remained significantly above the control level. The initial rise on exposure to -10°C was less and the fall during coma was greater in the twice- than in the once-exposed mice. Upon removal from the cold,

there was a tendency for the values to increase in both the once- and twice-stressed mice with PC levels tending to remain significantly elevated even after an hour of their return to the warmth. Within the first 6 hours after removal from the cold, the values were significantly lower in the twice-exposed than in the once-exposed mice, higher in the former on Day 1 and at the same level in both groups thereafter. Also, the variations in PC level during recovery were less in the twice-cooled mice.

Table 1 shows that the pattern of change in the PC level of the cold-exposed mice could not be explained on the basis of the normal diurnal variation occurring in the warmth. In support of this was the lack of a significant correlation coefficient between the PC levels of exposed and non-exposed mice sacrificed at the same times.

The significance of the PC levels for the assessment of the change in adrenocortical function on exposure to -10°C must await assessment of the other variables investigated in this study.

4.4. THE CHANGE IN THE DAILY AMOUNT OF UC OF CF_1 FEMALE MICE ON EXPOSURE TO -10°C

The UC values of mice exposed once or twice to -10°C had lower values than those of the controls but statistically

significant differences were obtained only in the twice-exposed animals (Table 6). Also, the animals exposed twice had significantly lower values than the animals that had been exposed only once. In general, also the values on the 7th day after exposure were higher than those of the first day but the differences were not statistically significant. The PC values of Day 1 and Day 7 of the once- and twice-cooled mice were back to normal in all cases except on Day 1 of twice-stressed mice where the values were somewhat elevated.

A further discussion of these data in the assessment of adrenocortical function must await assessment of the Hb, BUN and plasma creatinine data.

4.5. THE CHANGE IN THE Hb LEVELS OF CF_1 FEMALE MICE ON EXPOSURE TO -10°C

The Hb levels of mice exposed once to -10°C showed no significant change, but the mice exposed twice did, with a signficant drop in comatose animals. In general, animals exposed twice to -10°C had significantly higher values than those exposed only once. The Hb levels of both once and twice-exposed animals who became comatose dropped but differences were significant only in the twice-exposed animals.

The Hb levels were determined to gain information on the degree of hemoconcentration occurring in cold-stressed

mice and to assess its possible influence on the PC level. When a correlation coefficient was calculated between all the Hb and PC data of either the first or second exposure, it did not achieve statistical significance. Moreover, the drop in Hb level which occurred when the animals were comatose was only 4% and could not account for the 26% drop which occurred at the same time in PC level of once-exposed mice or the 45% drop which occurred in the PC level of twice-exposed animals. Thus, it was not possible to explain the increase in PC level in cold-stressed mice on the basis of hemoconcentration.

Hemoconcentration has been reported previously in the literature as occurring in cold exposed animals (Rodbard, Saki, Malin and Young, 1951; Everett and Matson, 1961).

4.6. THE CHANGE IN BUN LEVELS OF CF_1 MICE ON EXPOSURE TO -10°C

The BUN data of mice exposed once (Table 8) or twice to -10°C (Table 9) had elevated values when compared with control values shown in Table 8. In the once-stressed mice the values appeared to increase the longer the animals were in the cold up to 1 hour, to decrease during coma, to increase again to the maximum 4 hours after removal from the cold and to return to normal gradually thereafter. This pattern of change followed very closely the change seen in the PC level

and indeed the correlation between the PC and BUN levels was positive and highly significant.

In mice exposed twice to the cold, the levels generally were also elevated but the pattern of change with time was somewhat different than that seen in the once-exposed mice. Thus, there was no steady increase in the level up to 1 hour prior to collapse, no decline during collapse but an increase to the maximum value in the early hours following collapse did occur. Thereafter, the values tended to return to normal levels. This time there was no significant correlation between the PC and BUN. These data suggest that some impairment in renal function occurs especially soon after coma.

The significant positive correlation coefficients between the PC and BUN level suggest that at least part of the elevation of the PC levels in the cold was due to a decline in renal function. However, the maximum increase in the PC levels over the controls was over 500% and occurred after 1 hour in the cold, whereas the maximum increase in the BUN level was well below 200% and occurred 4 hours after removal from the cold when the PC levels had declined considerably. Moreover, during the second exposure, the extent of the increase in PC level was less than that of the first, but there was no significant change in BUN level. Further discussion of this point will be delayed until later.

4.7. CHANGE IN PLASMA CREATININE LEVELS OF CF_1 FEMALE MICE ON EXPOSURE TO -10°C

Unlike the BUN, the plasma creatinines showed an increase immediately on exposure to the cold but returned to normal by 1 hour after exposure. However, when the animals had become comatose and, indeed, up to 4 hours later when the animals had recovered, the plasma creatinine values were definitely elevated and returned to normal only the day following recovery. There was no significant correlation between the plasma creatinine and the PC level in mice exposed once or twice.

In animals whose two exposures were for 15 minutes or 1 hour the plasma creatinine levels were normal or even subnormal. But those whose first exposure was until coma all showed elevated values which remained in this state even 7 days following the second coma, the only exception being the group which was sacrificed after the second coma. Moreover, the values of mice exposed twice tended to be significantly higher than those exposed once. Nevertheless, the PC levels of mice exposed twice were not significantly higher than those exposed once; indeed, the reverse was true. Nor did the maxima of the PC levels correspond with those of the plasma creatinines during either exposure. Therefore, the decline in renal

function as shown by elevated plasma creatinine which did not occur until the mice became comatose could not have influenced the markedly elevated values prior to this time. Whether they could have influenced them later will be discussed in the next section. The BUN is an overall measure of renal function without measuring any single function specifically. On the other hand, the persistently high plasma creatinine levels following coma indicate that there may have been some damage to glomerular filtration, although more information would be required in terms of determinations of urinary creatinine.

4.8. THE PC, Hb, BUN AND PLASMA CREATININE LEVELS AND UC AMOUNTS IN MICE ONE AND SEVEN DAYS AFTER ONE OR TWO EXPOSURES TO -10°C (TABLE 11)

On the first day following the first exposure, the PC and Hb levels and UC amounts were normal while the BUN and plasma creatinine levels were elevated. This suggests that there was no hemoconcentration at this time and despite the decline in renal function suggested by the elevated BUN and plasma creatinine values, both the PC and UC levels were not markedly influenced.

Seven days following the first exposure, there appeared to be some slight hemoconcentration and renal function

Table 11. The PC, Hb, BUN and Plasma Creatinine Levels and UCS Amounts in Control and Cold-Stressed Mice Exposed Until Comatose Once or Twice to -10°C

Variable	No Exposure	One Exposure On		Two Exposures On	
		Day 1	Day 7	Day 1	Day 7
PC (mcg%)	9.2 ± 1.7	8.9 ± 4.1	5.9 ± 0.9	20.8 ± 3.0	6.3 ± 1.3
UC (μg/24 hours)	340 ± 72	293 ± 24	360 ± 52	174 ± 29	207 ± 46
Hb (g%)	13.2 ± 0.2	12.6 ± 0.6	13.9 ± 0.2	12.7 ± 0.4	14.5 ± 0.5
BUN (mg%)	24.0	28.0	16.6	20.0	21.2
Plasma Creatinine (mg%)	0.44	0.63	0.63	1.14	0.86

The mean and standard errors were calculated from the values obtained from 6 mice except in the case of the values under Day 7 of One-Exposure where 4 animals were involved. The BUN and plasma creatinine values were obtained from pooled plasma.

as a whole appeared normal as indicated by the BUN, though there appeared to be some abnormality in glomerular filtration as suggested by the elevated plasma creatinine. However, whatever hemoconcentration and renal impairment may have existed at this time, they were apparently insufficient to alter significantly either the PC level or the UC amounts, for they were at the normal level at this time.

On the first day following the second exposure to -10°C, the PC levels appeared elevated, the UC levels significantly depressed, the Hb and BUN levels normal and plasma creatinine definitely elevated. Thus, there was no hemoconcentration, but there appeared to be an impairment in glomerular filtration. This would account for the elevated PC level observed and for the decline in UC amounts observed at this time. Thus, it is not possible to make an accurate assessment of adrenal cortical function at this time due to interference of the renal impairment.

On the seventh day following the second exposure to -10°C, there was hemoconcentration, the BUN was normal, but the plasma creatinine was still definitely elevated. However, neither the hemoconcentration nor the impaired kidney function appeared to have markedly raised the PC level which was normal at this time. However, the UC values were somewhat subnormal at this time. It is possible that following Day 1 of the

second exposure to -10°C the elevated PC level depressed adrenocortical function and this could have carried over until Day 7, hence accounting for the depressed UC amounts at this time. The normal PC level in the presence of depressed adrenocortical function could be due to the renal impairment. That is, although the renal impairment did not markedly raise the PC level above normal, it may have increased it to normal from a subnormal level.

Analysis of variance showed that the UC amounts of Day 7 were significantly higher than those of Day 1 and this could have been due to an improvement in renal function within this time.The BUN levels declined on Day 7 as compared with Day 1 in the once-exposed mice and the plasma creatinine levels declined during the same time in the twice-exposed animals.

It is clear from these results that adrenal cortical function was certainly not elevated on Day 1 or Day 7 after 1 or 2 exposures to -10°C until the mice became comatose. Wherever elevated PC levels were observed at these times they were attributable to impairment in renal function and wherever UC levels were depressed they could also be accounted for in the same way. Examination of these data would, therefore, suggest that adrenal cortical function was perhaps subnormal at these times and at the most normal.

4.9. THE CHANGES IN PC LEVEL IN COMA

The data presented in this study indicated that PC levels were elevated in CF_1 female mice during a first and a second exposure to -10°C, the second elevation in PC level being somewhat less than that of the first, but still very high. During coma, at both time intervals, the values fell from their maximum values but were still well above the normal control levels. The finding of the elevated PC levels when the animals were comatose, that is, when their body temperatures were well below 20°C, points to the fact that the PC level is the resultant not only of its production by the adrenal cortex and of its rate of release from the gland, but also of the space in which it is distributed, its binding by CBG, its metabolism into other compounds and rate of excretion. Normally, an animal at 20°C or less would be expected to synthesize and excrete adrenal hormones at a much reduced rate and, indeed, this is supported by the finding of Hume and Egdahl (1959) who studied dogs rendered hypothermic. They demonstrated that as the temperature was dropped, the level of adrenal steroid in adrenal vein blood diminished and increased when the temperature was increased. These findings confirmed the earlier work of Ganong, Bernhard and McMurray (1955). Hume and Egdahl (1959) also found that when the animals were hypothermic, the adrenal cortex was not responsive to ACTH. The question then arises as to why in the hypothermic mice of our study the PC levels were not lower.

One possible explanation is that renal function was markedly depressed at this time preventing excretion of the circulating corticosterones. However, if this were so, it would have been expected that BUN and plasma creatinine values at this time would have been much more elevated than they actually were. On the contrary, the BUN values declined markedly during the first coma and the plasma creatinines showed that same change during the second coma. Obviously, the problem is more complex.

Inasmuch as several processes are involved in maintaining a certain PC level, the equilibrium which exists between them at normal body temperatures is very likely disturbed when the body temperature is markedly lowered. Nevertheless, this disturbance cannot be too far in any one direction for the PC level in the comatose mice still remains markedly elevated. This elevated PC level may be helpful to the animal to sustain the stress it undoubtedly undergoes in restoring its body temperature to normal when it is removed from the cold to the warmth, as indicated by the strong shivering it experiences as its temperature climbs back to normal. Further study of this process is indicated.

5. GENERAL DISCUSSION

A considerable body of data exists in the

literature on the changes in adrenal cortical function of animals exposed suddenly to the cold, most of which have been conducted in rats (Heroux and Schonbaum, 1959; Heroux, 1960; Denyes and Horwood, 1960; Schonbaum, 1960; and Boulouard, 1966). In all these studies, the rats were exposed to temperatures above 0°C and this togehter with the fact that their larger size renders them more resistant to the cold than mice indicates that the stress was less in the earlier studies than in the present one. For example, in one study, (Jonec, 1964) rats were exposed to 0°C for 6 exposures of one hour each. They reported that the steroid content of the adrenal and the plasma increased following cold exposure and that the effect was greatest during the first 3 exposures and gradually diminished by the sixth exposure at which time the values, however, were still above those of the controls. Jonec (1964) reported a maximum plasma corticosteroid level of 30 mcg%. This occurred after the first exposure and was less than that observed in our smaller animals exposed to a more acute temperature.

One study conducted in 2 subspecies of mice exposed to the cold (2 ± 0.5°C) for 15 days revealed that there was a significant rise in free and bound corticosterone in the plasma after cold exposure with a return to pre-exposure levels within 3 days. In the more responsive of the 2 subspecies (P.m. bairdii) the maximum increase in the PC level was 112%

whereas in the second subspecies (P.m. gracilis) the maximum increase was only 18%, a much smaller response than under the more severe conditions of our own study (Eleftheriou, 1964).

None of these studies, however, have investigated the adrenal cortical levels in animals kept in the cold until they became comatose nor did they investigate changes in circulating corticosterone following recovery. Furthermore, the studies described in this thesis also provided new information on what role hemoconcentration may have had in the elevation of the PC in the cold. Also, the studies on renal function indicated that some impairment had possibly occurred as a result of the coma temporarily after the first exposure but for a longer period of time following the second one and these must be kept in mind when attempting to assess the changes of PC level following exposure to the cold. Another feature of this study is that direct evidence was made available that mice exposed twice to the cold were significantly more resistant to it than mice exposed only once in that the time that it took them to become comatose was significantly longer the second time. This has been shown more extensively in earlier studies (Grad, Levine and Berenson, 1968). Thus, the increased resistance to the cold, as determined by the increased time taken to go into coma occurred despite the apparent decrease in renal function, and apparent decrease in responsiveness of the

PC level to the second cold exposure. Moreover, despite the apparent decline in renal function following 2 exposures to the cold, animals that had been exposed to the cold until coma 9 times were found not to suffer from a significant reduction in life span (Grad, Rafizadeh, Casoff and Berenson, 1969). Finally, these results tend to support the earlier claim that the adrenal steroids are essential only at the beginning of exposure to cold for Selye (1937) found that once rats had been adapted to the cold, removal of the adrenals no longer affected their resistance. Similar experiments were also observed by Gross and Lebland (1943) who found that for resistance of rats to the cold over long periods the presence of the thyroid was essential.

6. SUMMARY AND CONCLUSIONS

Twelve to 15 months old CF_1 female mice were exposed to -10°C for various time intervals up to and including the time when they became comatose as a result of the exposure. Other groups of mice were also exposed until they became comatose and for varying periods after removal from the cold to the warmth where the mice recovered from the coma. Some animals were exposed only once to the cold while others were exposed twice with a 7 day interval inbetween. Determinations of the PC, Hb, BUN and plasma creatinine levels and UC amounts were determined,

as were also colonic temperatures and body weights at appropriate times.

The results showed that the PC levels were elevated in mice exposed once or twice to the cold and although they dropped when the animals became comatose, the PC still remained reliably above normal at this time. On recovery from the cold, the values remained high for 6 hours in the once-stressed animals and up to and including the first day in the twice-exposed animals. In general the PC levels of mice exposed twice was lower than that of mice exposed once both during and after the exposure.

The UC amounts measured on the first and seventh day after the first and second exposures to -10°C were in general below those of the non-exposed controls, those of the second day being significantly so. The values on the seventh day were not significantly higher than those of the first.

The Hb levels showed no significant change on the first exposure to -10°C but showed a significant change on the second. The statistical significance was especially due to the decline in the Hb levels in mice at the time of their second coma in the cold. In general, also the Hb levels of the twice-stressed animals were also significantly higher than those of the animals stressed only once.

The BUN levels increased soon after the first exposure to the cold and remained elevated up until 4 hours following recovery from coma. Thereafter, they returned to normal levels. In animals exposed twice, the values tended to return to normal between 6 hours and 1 day after recovery from coma. There was no significant difference in the BUN of once- or twice-exposed mice.

Remarkable increases in the plasma creatinine were noted especially after the animals had become comatose. When the animals had become comatose after only one exposure, the values remained elevated for 4 hours following recovery from the coma. However, in animals that had been rendered comatose twice, the values remained elevated for at least 7 days after the second coma.

The results indicate that there is an increased adrenocortical function on exposure of the mice to the cold as manifested by elevated PC levels. This increase in PC level is not due to the hemoconcentration or impairment of renal function which was observed to have occurred during this time, and which in fact was more apparent during the second exposure to and recovery from the cold when the PC levels were not as high during and after the first exposure.

One and 7 days following exposure to the cold, the adrenal cortex no longer appears to be hyperfunctional despite the intensity and indeed, near lethal experience in the cold. Renal function appears to have been impaired for 4 hours following the first exposure and for at least 7 days after the second one. This impairment probably played an important role in maintaining the PC level and reducing the UC amounts on the first day following the second exposure.

BIBLIOGRAPHY

BIBLIOGRAPHY

ADDISON, T.:
London, Highley, 1855.

ADOLPH, E. F. and MOLNAR, G. W.:
Am. J. Physiol., 146:507, 1946.

ADOLPH, E. F.:
Am. J. Physiol., 163:695, 1950.

ASHWORTH, C. T., RACE, G. and MOLLENHAVER, H. H.:
Am. J. Path., 35:425, 1959.

AYRES, P. J., GOULD, R. P., SIMPSON, S. A. and TAIT, J. F.:
Biochem. J., 63:191, 1956.

BAIRD, P. C. Jr., CLONEY, E. and ALBRIGHT, F.:
Am. J. Physiol., 104:489, 1933.

BALL, E. G. and JUNGAS, R. L.:
Proc. Natl. Acad. Sci., 47:932, 1961.

BARNETT, S. A. and COLEMAN, E. M.:
J. Endocrin., 19:232, 1959.

BARTTER, F. C., LIDDLE, G. W., DUNCAN, L. E., BARBER, J. K. and DELEA, C. C.:
J. Clin. Invest., 35:1306, 1956.

BAUMANN, E. J. and KURLAND, S.:
J. Biol. Chem., 71:281, 1927.

BAUMBER, J. and DENYES, A.:
Am. J. Physiol., 205:905, 1963.

BENZINGER, T. H.:
Fed. Proc. Suppl. (5), 19:32, 1960.

BERGNER, G. E. and DEANE, H. W.:
Endocrinol., 43:240, 1948.

BERNARD, C.:
Baillière et Cie., Paris, 1876.

BIEDL, A.:
In "The Internal Secretory Organs: Their Physiology and Pathology", p. 606, Wm. Wood & Co., New York, 1913.

BIGGERS, J. D., ASHOUB, M. R., McLAREN, A. and MICHIE, D.:
J. Exp. Biol., 35:144, 1958.

BLIGH, J.:
Biol. Rev., 41:317, 1966.

BOULOUARD, R.:
Fed. Proc., 25:1195, 1966.

BOURNE, G. H. and JAYNE, E. P.:
"The Adrenal Gland", Bourne, G. H., (ed.)., pp. 307-324,
Hafner Publishing Co., Inc., New York, 1961.

BRAUN-MENENDEZ, E., FASCIOLO, J. C., LELOIR, L. F., MUNOZ, J. M.
and TAQUINI, A. S.:
"Renal Hypertension", Charles C. Thomas,
Springfield, Ill., 1946.

BROLIN, S. E.:
Acta. Anat., 2;1, 1946.

BROWN-SEQUARD, C. E.:
C. R. Acad. Sci., 43:422, 1856.

BURTON, A. C. and BRONK, D.:
Am. J. Physiol., 119:284, 1937.

BUSH, I. E.:
J. Physiol., 115:12, 1951.

CANNON, W. B., QUERIDO, A., BRITTON, S. W. and BRIGHT, E. M.:
Am. J. Physiol., 79:466, 1926-27.

CANNON, W. B.:
"The Wisdom of the Body", pp. 19-40, 2nd (ed.),
Norton, New York, 1939.

CARLSON, L. D.:
Fed. Proc. Suppl. (5), 19:25, 1960.

CARR, I.:
J. Path. Bact., 81:101, 1961.

CHAI, C. K. and DICKIE, M. M.:
In "The Biology of the Laboratory Mouse", p. 387,
Green, E. L. (ed.), McGraw-Hill, New York, 1966.

CLARK, R. T., Jr., CHINN, H. I., ELLIS, J. P., Jr., PAWEL, N. E. R.
and CRISCUOLO, D.:
Am. J. Physiol., 177:207, 1954.

COOK, W.:
Med. Chir. Trans., (London), 2:17, 1811.

COTTLE, W. H. and CARLSON, L. D.:
Proc. Soc. Exp. Biol. Med., 92:845, 1956.

COTTLE, W. H.:
Arctic Aeromed. Lab., Tech. Report 57-12, 1958.

COTTLE, W. H.:
Proc. Canad. Fed. Biol. Soc., 3:20, 1960.

CUSHING, H.:
Johns Hopk. Hosp. Bull., 50:137, 1932.

D'ANGELO, S. A.:
Fed. Proc. Suppl. (5), 19:51, 1960.

DAVIDSOHN, I. and WELLS, B. B.:
In "Clinical Diagnosis by Laboratory Methods", 13 (ed.), p. 73, Saunders & Co., 1963.

DAVIS, T. R. A., JOHNSTON, D. R., BELL, F. C. and CREMER, B. J.:
Am. J. Physiol., 198:471, 1960.

DAVIS, T. R. A. and MEYER, J.:
Am. J. Physiol., 181:675, 1955.

DEANE, H. W. and GREEP, R. O.:
Am. J. Anat. 79:117, 1946.

DEANE, H. W., SHAW, J. H. and GREEP, R. O.:
Endocrinol., 43:133, 1948.

DENNIS, C. and WOOD, E. H.:
Am. J. Physiol., 129:182, 1940.

DENNY-BROWN, D., GAYLOR, J. B. and UPRUS, V.:
Brain, 58:233, 1935.

DENYES, A. and HORWOOD, R.:
Canad. J. Biochem. Physiol., 38:1479, 1960.

DEPOCAS, F.:
Canad. J. Biochem. and Physiol., 38:107, 1960a.

DEPOCAS, F.:
Fed. Proc. Suppl. (5), 19:19, 1960b.

DEPOCAS, F.:
Brit. Med. Bull., 17:25, 1961.

DESMARAIS, A.:
Rev. Canad. Biol., 12:99, 1953.

DESMARAIS, A.:
Canad. J. Biochem. Physiol., 33:758, 1955.

DONALDSON, J. C.:
Mem. Wistar Inst. Anat., 2nd (ed.), p. 6, 1924.

DONTCHEFF, L. and SCHAEFFER, G.:
C. R. Soc. Biol., Paris, 127:1294, 1938.

DORFMAN, R. I.:
In "Adrenal Cortical Hormones", pp. 325-362,
Acad. Press Inc., New York, 1950.

DUGAL, L. P., SAUCIER, G. and DESMARAIS, A.:
Canad. J. Biochem. Physiol., 40:325, 1962.

ELEFTHERIOU, B. E.:
J. Endocrin., 31:75, 1964.

EULER, C. Von.:
Pharmacol. Rev., 13:361, 1961.

EVERETT, N. B. and MATSON, L.:
Arctic Aeromed. Lab., Project No. 8237-26, 1961.

EVERSOLE, W. J., GAUNT, R. and KENDALL, E. C.:
Am. J. Physiol., 135:378, 1942.

FASCIOLO, J. C., DE VITIO, E., ROMERO, J. C. and CUCCHI, J. N.:
Canad. Med. Assoc. J., 90:206, 1964.

FAVARGER, P. and GERLACH, J.:
Acta. 13:96, 1955.

FAVARGER, P.:
Exposés ann. de Biochim. Méd., 17:57, 1955.

FELTS, J. M. and MASORO, E. J.:
Am. J. Physiol., 197:34, 1959.

FIELD, J., 2nd, BELDING, H. S. and MARTIN, A. W.:
J. Cell. Comp. Physiol., 14:143, 1939.

FIRROR, W. M. and GROLLMAN, A.:
Am. J. Physiol., 103:686, 1933.

FORD, J. H. and YOUNG, R. W.:
Anat. Rec., 146:125, 1963.

FREDERICQ, L. A.:
Arch. de Biol., 3:687, 1882.

FREDERICQ, L. A.:
Arch. de Zool. Expér. et Gén.: iii, XXXV, 1885.

FREGLY, M. J., IAMPIETRO, P. F. and OTIS, A.B.:
J. Appl. Physiol., 16:127, 1961.

de FREITAS:
9th Canad. Cold Conf., Edmonton, 1967.

FRY, F. E. J.:
Publications of the Ontario Fish Research Laboratories, 1947.

GANONG, W. F., BERNHARD, W. F. and McMURRAY, J. D.:
Surg., 38:506, 1955.

GENEST, J., NOWACZYNSKI, W., KOIW, E., SANDOR, T. and BIRON, P.:
Canad. Med. Assoc. J., 84:403, 1961.

GENEST, J., VEYRAT, R., DE CHAMPLAIN, J., BOUCHER, R., TREMBLAY, G., STRONG, C. G., KOIW, E. and MARC-AURELE, J.:
Proc. of the Council for High Blood Pressure Research, 13:97, 1965.

GIROUD, G. J. P., STACHENKO, J. and VENNING, E. H.:
Proc. Soc. Exp. Biol. Med., 92:154, 1956.

GRAD, B. and KHALID, R.:
J. Gerontology, 23:522. 1968.

GRAD, B., LEVINE, J and BERENSON, J.:
XXIV International Congress of Physiological Sciences, 7:169, 1968.

GRAD, B., RAFIZADEH, F., CASSOFF, J. and BERENSON, J.:
8th International Congress of Gerontology, 1969.

GRAD, B. and KHALID, R.:
1969a (In preparation).

GRAD, B. and KHALID, R.:
1969b (In preparation).

GRAD, B., SANCHO, E. and MURPHY, B.E.P.:
1969 (In preparation).

GRANT, J.K.:
In "The Biosynthesis and Secretion of Adrenocortical Steroids", Clark, F. and Grant, K.J. (eds.), pp. 24-39, Camb. Univ. Press, Cambridge, 1960.

GREEP, R.O. and DEANE, H.W.:
Ann. N.Y. Acad. Sci., 50: 596, 1949.

GROLLMAN, A.:
In "The Adrenal Gland", pp. 286-288, Lippincott Co., Montreal, 1941.

HALBERG, F., PETERSON, R.E. and SILBER, R.H.:
Endocrinology, 64: 222, 1959.

HALE, H.B. and MEFFERD, R.B., Jr.:
Am. J. Physiol., 194:469, 1958.

HAMMEL, H.T.:
In "Handbook of Physiology", pp. 413-434, D.B. Dill (ed.), Am. Physiological Society, Washington, D.C., 1964.

HAMMEL, H.T., CALDWELL, F.T., Jr. and ABRAMS, R.M.:
Science, 156-1260, 1967.

HANNON, J.P.:
Proc. Soc. Exp. Biol. and Med., 97:368, 1958.

HANNON, J.P. and YOUNG, D.W.:
Am. J. Physiol., 197: 1008, 1959.

HANNON, J.P.:
Am. J. Physiol., 198:740, 1960.

HARDY, J.D.:
Physiol. Rev., 41:521, 1961.

HARDY, J.D., HELLON, R.F. AND SUTHERLAND, K.:
J. Physiol. (London), 175:242, 1964.

HART, J.S.:
Canad. J. Res., D 28:293, 1950a.

HART, J.S.:
Canad. J. Sool. 30:90, 1950b.

HART, J. S. and HEROUX, O.:
Canad. J. Biochem. Physiol., 33:428, 1955.

HART, J. S., HEROUX, O. and DEPOCAS, F.:
J. Appl. Physiol., 9:399, 1956a.

HART, J. S., HEROUX, O. and DEPOCAS, F.:
J. Appl. Physiol., 9:404, 1956b.

HART, J. S.:
Fed. Proc., 17:1045, 1958.

HART, J. S.:
In "Temperature-its measurement and control in Science and Industry", Vol. 3, pp. 373-406, C. M. Herzfeld (ed.), Reinhold, New York, 1963.

HART, J. S.:
Symp. Soc. Exp. Biol., 18:31, 1964a.

HART, J. S.:
In "Handbook of Physiology", D. B. Dill (ed.), Am. Physiological Society, Washington, D. C., 1964b.

HASTINGS, A. B. and COMPERE, E. L.:
Proc. Soc. Exp. Biol. Med., 28:376, 1931.

HATERIUS, H. O. and MAISON, G. L.:
U.S.A.F., MCREXD. 696-113, 5th Feb. 1948.

HAUSBERGER, F. X.:
Diabetes, 7:211, 1958.

HAYANO, M., SABA, N., DORFMAN, R. I. and HECHTER, O.:
Rec. Progr. Horm. Res., 12:79, 1956.

HEGNAUER, A. H. and PENROD, K. E.:
A.F. Tech. Report, 5912, Feb. 1950.

HENSEL, H.:
Recent advances in thermoreceptor physiology. In Proceedings, Symposia on Arctic Biology and Medicine. 1. Neural Aspects of Temperature Regulation. Arctic aeromed. Lab., 1961.

HEROUX, O. and HART, J. S.:
Am. J. Physiol., 178:449, 1954a.

HEROUX, O. and HART, J. S.:
Am. J. Physiol., 178:453, 1954b.

HEROUX, O.:
Am. J. Physiol., 181:75, 1955.

HEROUX, O. and SCHONBAUM, E.:
Canad. J. Biochem. Physiol., 37:1255, 1959.

HEROUX, O., SCHONBAUM, E. and DESMARAIS, A.:
Fed. Proc., 18:67, 1959.

HEROUX, O.:
Fed. Proc. Suppl. (5), 19:82, 1960.

HEROUX, O.:
Canad. J. Biochem. Physiol., 40:537, 1962.

HIMMS-HAGEN, J.:
Canad. J. Physiol. and Pharm., 43:379, 1965.

HOBERMAN, H. D.:
Yale J. Biol. and Med., 22:341, 1950.

HOLZBAUER, M.:
J. Physiol., 139:294, 1957.

HOUSSAY, B. A. and SAMMARTINO, R.:
Compt. Rend. Soc. de Biol., 114:1717, 1933a.

HSIEH, A. C. L. and CARLSON, L. D.:
Am. J. Physiol., 190:243, 1957.

HSIEH, A. C. L., CARLSON, L. D. and GRAY, G.:
Am. J. Physiol., 190:247, 1957.

HUME, D. M. and EGDHAL, R. H.:
Annals of the N.Y. Acad. of Sci., 80:435, 1959.

HUSTON, M. J. and MARTIN, A. W.:
Proc. Soc. Exp. Biol. and Med., 86:103, 1954.

IBANEZ, F. M.:
Internat. Rec. of Med., 165:547, 1952.

INGLE, D. J., MEEKS, R. C. and HUMPHREY, L. M.:
Am. J. Physiol., 173:387, 1953.

IRVING, L.:
Fed. Proc., 10:542, 1951.

JOEL, C. D. and SHACKNEY, S. E.:
Fed. Proc., 21:184, 1962.

JOEL, C. D., TREBLE, D. H. and BALL, E. G.:
Fed. Proc., 23:271, 1964.

JOHNEC, V.:
Canad. J. Physiol. Pharmacol.,42:585, 1964.

JONES, I. C. and ROBY, C. C.:
J. Endocr., 10:245, 1954.

JONES, I. C. (ed.):
"The Adrenal Cortex", Camb. Univ. Press, Cambridge, 1957.

KASS, E. H., HECHTER, O., MACCHI, I. A. and MOON, T. W.:
Proc. Soc. Exp. Biol. Med., 85:583, 1954.

KENDALL, E. C.:
Vitamins and Hormones, 6:277, 1949.

KENYON, A. T.:
Am. J. Pathol., 9:347, 1933.

KITAY, J. I., HOLUB, D. A. and JAILER, J. W.:
Endocrinol., 64:475, 1959.

KLEIBER, M.:
Proc. Soc. Exp. Biol. and Med., 48:419, 1941.

KLEIBER, M.:
Physiol. Rev., 27:511, 1947.

KLEIBER, M.:
"The Fire of Life", Wiley, New York, 1961.

KNIGGE, K. M. and BJERMAN, S. M.:
Am. J. Physiol., 192:625, 1958.

KONIG, F. H.:
Arch. ges. Physiol., 246:693, 1943.

KONIG, F. H.:
Pflügers Arch., 247:497, 1944.

KREBS, H.A.:
Biochem. et Biophys., Acta, 4:249, 1950.

LACHANCE, J. and PAGE, E.:
Endocrinology, 52:57, 1953.

LACHANCE, J. and PAGE, E.:
Rev. Canad. Biol., 14:74, 1955.

LANGLEY, L.L.:
In "Homeostasis", p. 24, Reinhold Publishing Corp., N.Y., London, 1965.

LASIEWSKI, R.C.:
Physiol. Zool., 36:122, 1963.

LATHE, G.H. and PETERS, R.A.:
Quart. J. Exp. Physiol., 35:55, 1949.

LEBLANC, J.A. and NADEAU, G.:
Canad. J. Biochem., 39:215, 1961.

LEBLOND, C.P. and GROSS, J.:
Endocrinol., 33:155, 1943.

LEBLOND, C.P., GROSS, J., PEACOCK, J. and EVANS, R.D.:
Am. J. Physiol., 140:671, 1944.

LEMONDE, P. and TIMIRAS, P.S.:
Rev. Canad. Biol., 10:76, 1951.

LEVIN, L.:
Am. J. Physiol., 138:577, 1943.

LEVINE, S. and TREIMAN, D.M.:
Endocrinol., 75:142, 1964.

LIBERMEISTER, K.:
Arch. Anat. u. Physiol., p. 520, 1860.

LIM, T.P.K.:
Fed. Proc., 18:93, 1959.

LOEB, R.F., ATCHLEY, D.W., BENEDICT, E.M. and LELAND, J.:
J. Exp. Med., 57:775, 1933.

LONG, C.N.A., KATZIN, B. and FRY, E.G.:
Endocrinol., 26:309, 1940.

LUTZ, W.:
Klin. Wchnschr. 22:727-733, 1943.

MARINE, D. and BAUMANN, E. J.:
Am. J. Physiol., 81:86, 1927.

MARTIN, A. W. and FUHRMAN, F. A.:
Physiol. Zool., 28:18, 1955.

MASON, J. W.:
In Jasper, H.H. et al (eds), Reticular Formation of the Brain, Boston: Little, Brown, 1958.

MASORO, E. J., COHEN, A. I. and PANAGOS, S. S.:
Am. J. Physiol., 179:451, 1954.

MASORO, E. J. and FELTS, J. M.:
Fed. Proc., 18:99, 1959a.

MASORO, E. J. and FELTS, J. M.:
J. Biol. Chem., 234; 198, 1959b.

MASORO, E. J., PORTER, E. and PATKIN, J.:
Arctic Aeromed. Lab., Project No. 8237-38, 1961.

MASORO, E. J.:
Fed. Proc., 22:868, 1963.

MATSUDA, K., DUYCK, C. and GREER, M. A.:
Endocrinol., 74:939, 1964.

MEFFERD, R. B., Jr., HALE, H. B. and MARTENS, H. H.:
Am. J. Physiol., 192:209, 1958.

MENDELSOHN, E.:
In "Heat and Life", University Press, Harvard, 1964.

MILSTEIN, S. W. and HAUSBERGER, F. X.:
Diabetes, 5:89, 1956.

MOEHLIG, R. C. and BATES, G. S.:
Arch. Internal. Med., 51:207, 1933.

MORIN, G.:
Rev. Canad. Biol., 5:388, 1946.

MURAKAMI, N.:
Am. J. Physiol., 213:1015, 1967.

MURPHY, B. E. P.:
J. Clin. Endo. & Metab., 27:973, 1967.

NICHOLLS, D., MOLLOY, R., STAVRAKY, K. and ROSSITER, R. J.:
Canad. J. Biochem. and Physiol., 37:671, 1959.

NOELL, W.:
Arch. Psychiat., 118:29, 1945.

OLIVER, G. and SCHAEFER, E. A.:
J. Physiol., 18:276, 1894-1895.

PAGE, E. and BABINEAU, L. M.:
Rev. Canad. Biol., 9:202, 1950.

PAGE, E., BABINEAU, L. M. and LACHANCE, J. P.:
Rev. Canad. Biol., 14:144, 1955.

PAGE, E.:
Rev. Canad. Biol., 16:269, 1957.

PATKIN, J. K. and MASORO, E. J.:
Am. J. Physiol., 200:847, 1961.

PATKIN, J. H. and MASORO, E. J.:
Canad. J. Physiol. Pharmacol., 42:101, 1964.

PERRAULT, M. J. and DUGAL, L. P.:
Canad. J. Biochem. Physiol., 40:1717, 1962.

PERRAULT, M. J. and DUGAL, L. P.:
Rev. Canad.Biol., 22:77, 1963.

PERRAULT, M. J. and DUGAL, L. P.:
Canad. J. Physiol. Pharmacol., 43; 809, 1965.

PFLUGER, E. F. W.:
Pflüger's Arch., XV:57, 1877.

PORGES, O.:
Ztschr. J. Klin. Med., 69:341, 1910.

RAND, C. G., RIGGS, D. S. and TALBOT, N. B.:
Endocrinology, 51:562, 1952.

REICHSTEIN, T. and SCHOPPEE, C. W.:
Vitamins & Hormones, 1:345, 1943.

REINER, M.:
In "Standard Methods of Clinical Chemistry", Vol. 1, p. 119, Academic Press, N. Y., 1953.

REINER, M.:
In "Standard Methods of Clinical Chemistry", Vol. 1, p. 55, Academic Press, N.Y., 1953.

RICHET, C.:
Dictionnaire de Physiologie, Paris, iv:72.

RICHET, C.:
Compt. Rend. Soc. de Biol., 44:896, 1892.

RIEDESEL, M. L.:
Trans. Kans. Acad. Sci., 60:99, 1957.

RIGLER, R.:
Klin. Wchnschr., 14:1, 1935.

RING, G. C.:
Am. J. Physiol., 137:582, 1942.

ROBERTS, S.:
J. Biol. Chem., 200:77, 1952.

ROBINSON, S., EDWARDS, A. T. and DILL, D. B.:
Science, 85:409, 1937.

ROCHEFORT, G. J., ROSENBERGER, J. and SAFFRAN, M.:
J. Physiol., 146:105, 1959.

RODBARD, S., SAIKI, H., MALIN, A. and YOUNG, C.:
Am. J. Physiol., 167:485, 1951.

ROGOFF, J. M.:
J. Am. Med. Assoc., 99:1309, 1932.

ROHRIG, A., ZUNTZ, N.:
Pflüger Arch. Ges. Physiol., 4:57, 1871.

ROSENTHAL, J., BOUCHER, R., NOWACZYNSKI, W. and GENEST, J.:
Canad. J. Physiol. Pharmacol., 46:85, 1968.

ROSSITER, R. J. and NICHOLLS, D.:
Rev. Canad. Biol., 16:249, 1957.

ROWNTREE, L. G. and SNELL, A. M.:
"A Clinical Study of Addison's Disease,"
W. B. Saunders Co., Phila., 1931.

SARASON, E. L.:
Arch. Path., 35:373, 1943.

SAYERS, G., SAYERS, M. A., FRY, E. G., WHITE, A. and LONG, C.N.H.:
Yale J. Biol. Med., 16:361, 1944a.

SAYERS, G., SAYERS, M. A., LEWIS, H. L. and LONG, C. N. H.:
Proc. Soc. Exp. Biol. Med., 55:238, 1944b.

SAYERS, G., SAYERS, M. A., LEWIS, H. L. and LONG, C. N. H.:
Endocrinol., 40:265, 1947.

SAYERS, G.:
Physiol. Rec., 30:241, 1950.

SCHAYER, R. W.:
Am. J. Physiol., 198:1187, 1960.

SCHOLANDER, P. F., WALTERS, V., HOCK, R. and IRVING, L.:
Biol. Bull., 99:225, 1950.

SCHOLANDER, P. F., HOCK, R., WALTERS, V., JOHNSON, F. and
IRVING, L.:
Biol. Bull., 99:237, 1950.

SCHOLANDER, P. F., WALTERS, V., HOCK, R. and IRVINC, L.:
Biol. Bull., 99:259, 1950.

SCHOLANDER, P. F.:
"A Principle in Biology", Aschehang and Co., Oslo, 1958.

SCHONBAUM, E.:
Fed. Proc. Suppl. (5), 19:85, 1960.

SELLERS, E. A. and YOU, R. W.:
Science, 110:713, 1949.

SELLERS, E. A. and YOU, S. S.:
Am. J. Physiol., 163:81, 1950.

SELLERS, E. A., YOU, S. S. and THOMAS, N.:
Am. J. Physiol., 165:431, 1951.

SELLERS, E. A. and YOU, R. W.:
Biochem. J., 51:573, 1952.

SELLERS, E. A., SCOTT, J. W. and THOMAS, N.:
Am. J. Physiol., 177:372, 1954.

SELLERS, E. A.:
Rev. Canad. Biol., 16:175, 1957.

SELYE, H.:
Canada Med. Assoc. J., 34:706, 1936.

SELYE, H.:
Ann. Physiol. Pharm. Int., 55:431, 1937.

SELYE, H.:
J. Clin. Endocrinol., 6:117, 1946.

SELYE, H. and TIMIRAS, P. S.:
Nature, 164:745, 1949.

SHORT, R. V.:
Symp. Biochem. Soc., 18:59, 1960.

SIMPSON, S. A.:
Quart. J. Med., 1:99, 1932.

SIMPSON, S. A,, TAIT, J. F., WETTSTEIN, A., NEHER, R.,
Von EUW, J., SCHINDLER, O. and REICHSTEIN , T.:
Experientia, 10:132, 1954.

SMITH, A. U.:
In "Biological Effects of Freezing and
Supercooling", pp. 304-364, Edward Arnold (Publisher)
Ltd., London, 1961.

SMITH, P. E.:
J. Am. Med. Assoc., 88:158, 1927.

SMITH, P. E.:
Am. J. Anat., 45:205, 1930.

SMITH, R. E. and HOIJER, D.:
Physiol. Rev., 42:60, 1962.

SMITH, R. E.:
Fed. Proc., 22:738, 1963.

SMITH, R. E. and ROBERTS, J. C.:
Am. J. Physiol., 206:143, 1964.

SNEDECOR, G.W.:
In "Statistical Methods", 5th (ed.), p. 291,
The Iowa State University Press, 1966.

SOFFER, L.J., DORFMAN, R.I. and GABRILOVE, J.L.:
"The Human Adrenal Gland", (Lee & Febiger), 1961.

SOLEM, J.H.:
Scand. J. Clin. lab. Invest., 18:1, 1966.

STARR, P. and ROSKELLEY, R.:
Am. J. Physiol., 130:549, 1940.

STEIN, L. and WERTHEIMER, E.:
Proc. Soc. Exp. Biol. Med., 46:172, 1942.

STEVENS, C.E., D'ANGELO, S.A., PASCHKIS, K.E.,
CANTAROW, A. and SUDERMAN, F.W.:
Endocrinology, 56:143, 1955.

SWANN, H.G.:
Physiol. Rev., 20:493, 1940.

SWANSON, H.E.:
Endocrinology, 60:205, 1957.

SWINGLE, W.W. and REMINGTON, J.W.:
Physiol. Rev., 24:89, 1944.

SYMINGTON, T. (ed.):
In "Mondern trends in pathology", p. 248,
Butterworth, London, 1959.

SYMINGTON, T.:
Biochem. Soc. Sympos., 18:40, 1960.

SYMINGTON, T.:
Brit. Med. Bull., 18:117, 1962a.

SYMINGTON, T.:
In "The Human Adrenal Cortex", Currie, A.R.,
Symington, R. and Grant, J.K. (eds.), Livingstone,
Edinburgh, London, 1962.

THOMPSON, G.E. and STEVENSON, J.A.F.:
Canad. J. Physiol. Pharmacol., 43:279, 1965.

THOMPSON. K.W. and EISENHARDT, L.:
J. Clin. Endocrinol., 3:445, 1943.

TRAPANI, I. L. and CAMPBELL, D. H.:
J. Appl. Physiol., 14:424, 1959.

TRAPANI, I. L.:
Fed. Proc., Suppl. (5), 19:109, 1960.

TREADWELL, C. R., FLICK, D. F. and VAHOUNY, G. V.:
J. Nutrition, 63:611, 1957.

TREADWELL, C. R., FLICK, D. F. and VAHOUNY, G. V.:
Proc. Soc. Exp. Biol. & Med., 97:434, 1958.

TREICHLER, R. and MITCHELL, H. H.:
J. Nutrition, 22:333, 1941.

TRILLER, H. and BIRMINGHAM, M. K.:
Gen. Comp. Endocr., 5:618, 1965.

TYSLOWITZ, R. and ASTWOOD, E. B.:
Am. J. Physiol., 136:22, 1942.

URQUHART, J., DAVIS, J. O. and HIGGINS, J. J. Jr.:
Physiologist, 5:224, 1962.

VAUGHAN, D. A., VAUGHAN, L. N. and KLAIN, G. J.:
J. Nutrition, 76:291, 1962.

VULPIAN, A.:
Compt. Rend. Acad. Sci. (Paris), 43:663, 1856.

WAITES, G. M. H. and MOULE, G. R.:
J. Reprod. Fert., 2:213, 1961.

WALTER, K., SELDIN, D. W. and BURNETT, C. H.:
Am. J. Med., 18; 454, 1955.

WEISS, A. K.:
Am. J. Physiol., 177:201, 1954.

WEISS, A. K.:
Fed. Proc., 15:197, 1956.

WELLS, B. B. and KENDALL, E. C.:
Proc. Staff Meeting Mayo Clinic, 15:3241, 1940.

WERTHEIMER, E. and BENTOR, V.:
Gerontologia, 1:306, 1957.

WEYMOUTH, F. W., FIELD, J. 2nd and KLEIBER, M.:
Proc. Soc. Exp. Biol. & Med., 49:367, 1942.

WILLIAMS, J. N., SCHURR, P. E. and ELVEHJEM, C. A.:
J. Biol. Chem., 182:55, 1950.

WILLIAMS, R. H.:
"Textbook of Endocrinology", Saunders Co., 1962.

WINEGRAD, A. I. and RENOLD, A. E.:
J. Biol. Chem., 233:267, 1958.

WINER, B. J.:
In"Statistical Principles in Experimental Design",
p. 298, McGraw-Hill Co., 1962.

WINKLER, A. W., HOFF, H. E. and SMITH, P. K.:
Proc. Am. Physiol. Soc., 306, 1941.

WIT, A. and WANG, S. C.:
Fed. Proc., 26:1672, 1967.

WOLF, O. and GREEP, R. O.:
Proc. Soc. Exp. Biol. & Med., 36:856, 1937.

YATES, F. E. and URQUHART, J.:
Physiol. Rev., 42:359, 1962.

YOU, R. W. and SELLERS, S. E.:
Endocrinology, 49:374, 1951.

YOU, S. S. YOU, R. W. and SELLERS, E. A.:
Endocrinology, 47:156, 1950.

YOUNG, D. R. and COOK, S. F.:
Proc. Soc. Exp. Biol. & Med., 89:482, 1955.