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Mineral metabolism and microcirculation in the hibernating hamster

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Dissertation

MINERAL METABOLISM AND MICROCIRCULATION

IN THE HIBERNATING HAMSTER

by

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I. INTRODUCTION

Many important and interesting adaptations of animals to unfavorable environmental conditions have been described. Some protozoa develop cysts, many insects pupate, the lungfishes burrow into the mud and secrete gelatinous capsules about themselves for protection against dessication during the dry season (Foster et al, 1939). There is a basic ecological division between animals whose body temperatures approximate the temperatures of their environments, the poikilotherms, and those animals that maintain an essentially constant body temperature, the "warm-blooded" or homoiothermic birds and mammals. Of the million or so known species of animals, all but about twenty thousand are poikilothermal. The distinction between poikilotherms and homoiotherms is not hard and fast. Honeybees and other social insects have partial control over their immediate microclimate and have become facultative homoiotherms as a result of social activities. Monotremes are transitional between poikilothermy and homoiothermy and show considerable variations in their body temperatures under different environmental conditions. Nestling birds may start as poikilotherms and later develop the ability to regulate their temperatures (Allee et al, 1949).

Hibernation in mammals is a seasonal defense mechanism in which homoiothermic forms become practically poikilothermic. This release of hibernators from the necessity of maintaining

body temperature at a high level, enables them to survive long periods of low temperature and decreased availability of food and water. Aestivation is a similar period of dormancy to escape heat and drought, and is found in many invertebrates and some mammals, particularly desert species. The columbian ground squirrel and a few other mammals both aestivate and hibernate. True hibernation has been reported in the mammalian orders Monotremata, Marsupialia, Insectivora, Chiroptera, and Rodentia. Hibernation of carnivores differs in important respects from that of other orders (Allee et al, 1949), and is known as pseudohibernation.

The earliest published observations upon physiological changes in hibernating animals were made by Spallanzani, and his interest in this phenomenon led him to keep individuals of all the known Italian species of hibernating mammals in his home for observation and experiment (Spallanzani 1787). Spallanzani particularly studied the respiratory changes which took place during hibernation and he wrote that a dormant marmot could be suspended in an atmosphere of carbon dioxide for four hours without ill effect but that when the experiment was repeated with a bird, the bird died immediately. Spallanzani cited this result as an example of the very small respiratory requirements of hibernating animals.

Since the time of Spallanzani, many investigators have proposed an enormous number of theories to explain hibernation (Rasmussen 1916), but confirmation has been lacking and the cause of hibernation is still not understood.

The typical hibernator

Hibernating mammals have been characterized as animals which depend upon insects and grasses for their chief food sources, live mostly in frozen underground burrows during the cold winter months, are able to tolerate cold, have an unstable temperature regulating mechanism, show a striking increase in body weight during the summer months and are sexually inactive during the winter (Foster et al 1939).

A test alleged to detect a potential hibernator consists of immersing the animal to the neck in cold water. Under these conditions, potential hibernators survive at a body temperature a few degrees above the freezing point of water while non-hibernators die if the body temperature falls below 19°C . The test is consistent with the fact that most non-hibernators have a lethal colonic temperature of about 20°C while hibernators generally have a much lower lethal temperature. The lethal colonic temperature of the golden hamster is 3.8°C . (Adolph et al, 1951).

Physiological changes associated with the induction of hibernation

Carbon Dioxide content of the blood. A high blood carbon dioxide content has been found in hibernating animals (Dubois 1895, McBirnie et al, 1953). The anesthetic effect of high concentrations of carbon dioxide is well known and Dubois theorized that autonarcosis by carbon dioxide excess was a cause of hibernation. However, other workers find little evidence that the respiratory center is depressed in hibernating animals. Although the

total carbon dioxide is higher during dormancy, the carbon dioxide tension of the blood is actually lower than normal and the bicarbonate and pH values are essentially the same in both hibernating and active hamsters (Lyman and Hastings 1951).

Blood serum magnesium. The anesthetic effect of the magnesium ion has been established for invertebrates (Tullberg 1892) and vertebrates (Meltzer and Auer 1908). The magnesium effect can be reversed by a trace of calcium. Magnesium decreases cell permeability and protoplasmic viscosity. Magnesium ions also decrease nerve conductivity possibly by interfering with the release of acetylcholine, the presumptive synaptic transmitter (Hutter and Kostial 1953). The central thermoregulatory mechanism may also be depressed by magnesium (Hall and Ellis 1951). Increase in the amount of magnesium in the blood serum may be an important factor in the induction of the torpor and hypothermia characteristic of hibernation. An increased concentration of magnesium in the blood serum has been reported in the hibernating hedgehog (Suomalainen 1938), groundhog (McBirnie et al, 1953), bat, thirteen lined ground squirrel, golden hamster, and black bear (Folk et al, 1956).

Blood sugar. Decreased amounts of sugar in the blood serum have been noted in hibernating animals. This is true for the marmot (Dubois 1896), hedgehog (Suomalainen 1938), woodchuck (McBirnie et al 1953), and golden hamster (Maynard and Torres 1956). Claude Bernard noticed that the livers of hibernators

showed a high content of glycogen before hibernation took place (Bernard 1881). Likewise, recent observations have shown that the majority of liver parenchyma cells of active ground squirrels contain a heavy concentration of glycogen granules while the corresponding cells of hibernating animals lack glycogen granules (Mayer and Bernick 1956). These reports suggest that a decrease of sugar in the blood is a factor in inducing hibernation. On the other hand, other investigators claim that the hibernating marmot (Endres 1930), the arctic ground squirrel (Musacchia and Wilber 1952), and the golden hamster (Lyman and Leduc 1953) have a normal or slightly higher than normal concentration of sugar in the blood while hibernating.

Hormonal balance. Changes in the endocrine glands, particularly in the pituitary and adrenals, have been observed in hibernating mammals and a number of investigators believe that hibernators have an annual activity cycle of the endocrines which is marked by autumnal involution and vernal hyperactivity (Britton 1928, Kayser 1950). Doctor Harvey Cushing noted that hibernation is similar in some respects to the results of hypopituitarism and experimental hypophyseal deficiency (Cushing and Goetsch 1913). Cushing and his associates studied hibernating woodchucks and observed that the pituitary glands of the hibernating animals were diminished in size and that the cells of the pars anterior lost their characteristic differential

reactions to acid and basic stains, while at the end of the period of dormancy the pituitaries enlarged and the differential staining reaction returned. Foster has stated that hypophysectomy induced hibernation in the thirteen lined ground squirrel, but that these animals died while in the hibernating state (Foster et al, 1939).

Cushing and Goetsch (1913) also reported that the cortex and medulla of the adrenal glands of their hibernating woodchucks contained significantly smaller cells than those of alert animals, and Foster has observed that the adrenal glands of hibernating woodchucks were atrophied (Foster et al, 1939). Moreover, both the spermophile (Mann 1916) and the thirteen lined ground squirrel (Foster 1939) are said to hibernate following adrenalectomy. On the other hand, a more recent report claims that the ground squirrel requires at least one-sixth of an adrenal gland for hibernation (Vidovic and Popovic 1954).

Deane and Lyman (1954) have compared the histology and histochemistry of the adrenal cortex of normal alert hamsters with that of hibernating animals. They stated that the zona glomerulosa of the hibernant was broader and fattier, implying greater activity than the corresponding zone of the adrenal cortex of control animals. Demers (1959) confirmed these results and, in addition, found evidence that the zona fasciculata of hibernating hamsters was less active than that of warm room controls.

The syndrome which follows adrenal insufficiency and the physiological changes coincident with hibernation, have many

striking similarities and suggest a possible role of the adrenal gland in the induction of the hibernating state. Adrenal insufficiency results in hemoconcentration, decreased blood pressure, decreased blood flow, decreased sodium, chloride, bicarbonate and glucose in the blood serum and increased potassium and non-protein nitrogen in the blood serum (Ingle 1944). Moreover, the capillaries of adrenalectomized animals become atonic and dilated causing a pooling of the blood with stasis and there is a bradycardia, a fall in body temperature and a lower respiratory quotient (Noble 1950).

Neurological changes. The central nervous system is the basic controlling mechanism of hibernation according to some investigators (Prosser 1950, Lyman and Chatfield 1955). These workers claim that the nervous system reacts differently to cold stimulation in hibernators as compared with non-hibernators.

Recent studies upon the mechanism for heat production and conservation in mammals show that the characteristic responses to cold exposure depend upon the discharge of the posterior hypothalamus via sympathetic and somatic outflows. These changes are widespread and include vasoconstriction in the skin, piloerection, shivering, elevation of the blood sugar and rise in the basal metabolic rate. It has been found experimentally that small isolated lesions of the posterior hypothalamus cause an animal to become poikilothermic. Men with destruction of the posterior hypothalamus by tumor or infection are thereby rendered incapable

of maintaining body temperature (Fulton 1955). These discoveries suggest the possibility of hypothalamic involvement in the mechanism of hibernation. The fact that the barbiturates lower body temperature and specifically suppress the hypothalamic nuclei is also pertinent.

Experimental evidence bearing upon the nervous mechanisms of hibernation is quite sparse. The presence of the mid-brain appears to be necessary for awakening from hibernation, but destruction of the cerebrum or corpora quadrigemina do not prevent arousal in the marmot (Dubois 1896). The first sign of electrical activity in the awakening hamster appears in the hippocampus and its various subcortical projections (limbic area), but the neocortex responds much later (Chatfield and Lyman 1954). The limbic system is a visceral brain controlling autonomic activities. It seems reasonable that it should trigger the rewarming process which occurs during arousal. However, the part played by the nervous system in the induction of hibernation remains unknown.

The hypothalamic control of the secretion of its trophic hormones by the pituitary appears to be established (Turner 1955), and section of the hypophyseal stalk in rats abolishes the activation of the thyroid in response to cold in this species (Brolin 1945). Since non-hibernators respond to cold stress by increased metabolism while the same type of stimulus results in eventual fall in metabolism in hibernators, the latter may well have a

nervous-pituitary-adrenal-thyroid complex of decreased sensitivity. Incidental observations have shown that hamster nerve conducts at temperatures as low as 3.4°C while the nerve of a non-hibernator (rat) failed to conduct below 9°C (Chatfield 1948).

Physiological changes which occur during hibernation

Body temperature. The body temperatures of hibernating mammals are very low. Prairie dogs were partly torpid at a body temperature of 22°C , and cheek pouch and rectal temperatures of ground squirrels remained only 0.5°C to 1.0°C higher than the cold room temperatures of 4.9°C to 8.4°C . The average temperature of the hibernating woodchuck was about 4.6°C and that of dormant bats 1.2°C (Johnson 1931). The hibernating hamster is reported to have a body temperature of less than 0.5°C above that of a cold room maintained between 5°C and 6°C (Lyman and Chatfield, 1955). In another series, the temperature of the hibernating hamster ranged between 7°C and 11°C (Suomalainen and Granstrom 1955). The temperature of the hibernating groundhog was between 5°C and 13°C (McBirnie et al 1953). The Chinese hedgehog in the hibernating state had a body temperature about 1°C above ambient (Chao and Yeh, 1950) and the hibernating arctic ground squirrel had a temperature of from 2°C to 3°C (Erikson 1956). It appears that all mammals have a body temperature of less than 13°C when in the state of deep hibernation (Kayser 1950).

Metabolism. Non-hibernators exposed to cold usually respond by increasing their metabolic rates presumably under the influence of thyroid hyperactivity (Ring 1942), and the daily requirements of young rats for thyroxine is greatly increased by cold exposure (Dempsey and Astwood 1943). Acute exposure to cold tripled the uptake of radioactive iodine in this species (Gross et al 1943). All non-hibernators tested seem to respond to cold exposure in a characteristic way, by increasing metabolism to support the active processes of heat production and conservation. The hibernator, on the other hand, reacts to cold exposure by an eventual decrease in metabolism which may be gradual, as in most species, or abrupt, as in the hamster (Lyman and Chatfield 1955). In any event, the metabolic rate drops to a value between one-thirtieth and one-hundredth of that in the resting state (Benedict and Lee 1938). The fuel used to support hibernation seems to be fat. This is indicated by the fact that the respiratory quotient is very close to 0.7 in those hibernating mammals studied (Kayser 1949, Musacchia and Wilber 1952). However, a drop in environmental temperature from 5°C to 0°C caused hibernating hamsters to double or triple their metabolic rates and generally to maintain their body temperatures about 3°C higher than the environment (Lyman 1948).

Circulation. The heart rate of the normal hamster anesthetized with nembutal is 350-450 per minute. During hibernation, the heart slows to from 4 to 21 beats per minute and

respiratory movements occur only sporadically. A-V dissociation and other cardiac irregularities appear in the electrocardiogram taken early in the arousal process and are presumed to exist during hibernation. (Lyman and Chatfield 1950).

Blood pressures have not been taken during actual hibernation since the procedure inevitably initiates the arousal mechanism. The blood pressure of the hamster in the very early stages of awakening is about 70/40 millimeters of mercury. The non-hibernating hamster, under light nembutal anesthesia, has a mean blood pressure of between 120 and 170 millimeters of mercury.

Various changes in the circulating blood have been noted in hibernating mammals. Prolongation of blood clotting time has been seen in hibernating hedgehogs (Suomalainen and Lehto 1952), ground squirrels (Svihla et al 1951), hamsters (Svihla et al 1952), and bats (Smith et al 1954).

Changes in the distribution of formed elements of the circulating blood associated with the hibernating state include eosinopenia in the hedgehog (Peter 1935), lymphopenia and neutrocytosis in the European hamster (Rath 1953) and golden hamster (Suomalainen and Granstrom 1955). The hemaglobin content and erythrocyte number are increased in the hibernating marmot (Dubois 1895), European hamster (Rath 1953), and golden hamster (Suomalainen and Granstrom 1955).

Among the few descriptions of the peripheral circulation of hibernating mammals is a report on the mesenteric circulation

in the dormant groundhog (McBirnie et al 1953). Few details were given, but no tendencies toward intravascular agglutination or vascular stasis were noted. Lyman and his associates have suggested that the bright pink paws of the hibernating hamster indicate a freely flowing circulation at that level, while the paling of the paws at the start of arousal suggests an accompanying vasoconstriction. Furthermore, these workers injected thorotrast into hamsters coming out of hibernation and subsequent X-ray examination showed differential vasoconstriction taking place in the visceral and hind leg areas and persisting until the body temperature approached normal. The cheek pouch and heart regions were observed to rewarm quickly while mid-abdominal and posterior body areas lagged behind and did not reach equilibrium temperature until the final stages of arousal (Lyman and Chatfield 1950). This confirms earlier findings (Dubois 1896) that the posterior body regions of mammals emerging from hibernation show considerable delay in rewarming as compared with the anterior regions.

Mast cells. Mast cells are unicellular endocrines which manufacture and release heparin, histamine and serotonin. They are widely distributed in connective tissue and are frequently found in the neighborhood of blood vessels (Fulton et al 1957). The presumed release of vasoactive substances by mast cells has influenced workers to study these cells in hibernating animals. It has been noted that hibernating mammals have a slow circulation, but despite this, their vital organs maintain an essential

blood supply and thrombi do not occur. In addition, both histamine and serotonin have been observed to have a hypothermic effect when injected into rats (LeBlanc and Rosenberg 1957a), and serotonin release may also assist in producing the torpidity of hibernation. Comparisons of numbers of mast cells in various sites of hibernating and active animals have shown increases in the thymus of the hibernating hedgehog (Peter 1935) and in the small intestine and bronchioles of the same species (Harma and Suomalainen 1951). The increased numbers of mast cells have been correlated with the lengthened blood clotting time of hibernating hedgehogs (Suomalainen and Lehto 1952). Increased blood clotting times have also been reported for the hibernating hamster (Svihla et al 1952), but counts of mast cells were not made. On the other hand, counts of mast cells of duodenal sections and blood clotting times of summer and winter bats failed to show a consistent relationship. The number of mast cells has been reported to increase in abdominal skin and mesentery of rats exposed to cold (LeBlanc and Rosenberg 1957b). Magnesium deficiency resulted in a fifty percent reduction of dermal mast cells in rats (Belanger et al 1957), but there are no reports relating mast cell numbers to the increased magnesium content of the blood serum that is supposed to occur in hibernating animals.

Arousal from hibernation

Arousal from hibernation has been described as a "coordinated physiological event involving a mass discharge of those parts of the nervous system governing heat production and conservation, resulting in activity of the sympathetico-adrenal and somatic motor systems" (Lyman and Chatfield 1955). The sequence of events taking place during the arousal process has been described (Dubois 1896, Johnson 1931, Lyman and Chatfield 1950). Arousal may be initiated by any one of a number of types of stimuli, and various mammalian species seem to react differently in this regard. Hibernating ground squirrels either awoke or died when the environmental temperature fell to -10°C and an increase in temperature to about 10°C , noise, or handling had a tendency to start the arousal process (Erikson 1956). The hamster is aroused by handling or by being removed to a warm room, but evidently does not respond to sound stimuli, nor could an electrical response be obtained from the round window at a temperature below 18°C (Kahana et al, 1950). Hibernating woodchucks are apathetic to touch stimulation but may be awakened by noises. This species shows a cortical potential in response to sound stimulation at cortical temperatures as low as 7°C (Lyman and Chatfield 1953).

Periodic awakenings during which the hamster fed and voided took place as often as once a week in Lyman's animals, and the longest continuous period of hibernation was 21 days. This is

contrary to what happens under natural conditions in most hibernating species (Johnson 1931). Lyman has stated that once the process of arousal starts in the hamster, it continues inevitably unless death intervenes. Arousal commences with spasmodic muscular twitching involving first the vibrissae and fore paws and gradually increasing in strength and extent, spreading to the entire anterior end of the animal and finally to the posterior end. These muscular movements are accompanied by increased cardiac and respiratory rates and increased cheek pouch and rectal temperatures.

It has been mentioned above that the mid-brain, but not the cerebrum or corpora quadrigemina are necessary for arousal (Dubois 1896). In artificially cooled cats, the adrenals seem necessary for awakening (Johnson 1931), but the hamsters adrenalectomized in the first five minutes of the waking period carried out the arousal process to completion (Lyman and Chatfield 1955). This was taken as evidence that arousal is not an example of Selye's "alarm reaction". Complete arousal required about two hours in the cold room according to Lyman. Most physiological studies of hibernation are really made during early arousal since the procedures involved almost always initiate the arousal process.

Artificial hibernation

The term "artificial hibernation" was originally used to describe the state of induced generalized hypothermia (Simpson

and Herring 1905). Artificial hibernation has been induced in woodchucks by placing them in an ice-cooled chamber at temperatures between 6°C and 13°C subsequent to injection of 25 units of insulin per kilogram of body weight. The woodchucks became completely dormant with body temperatures as low as 11.3°C and were maintained in that state for three days. Dormancy was abolished by the subcutaneous injection of 2cc of 1/1000 adrenaline. Controls maintained a body temperature of 37.3°C without appreciable shivering (Dworkin and Finney 1927).

Hedgehogs injected subcutaneously with molar magnesium chloride went into a "cold blooded state" which could be reversed by injection of molar calcium chloride (Suomalainen 1938) and magnesium chloride plus insulin were even more effective. Artificial hibernation has important applications in surgery. Patients being treated for neoplasms by refrigeration could be cooled more readily by deep narcotization prior to cold exposure. The anesthetics used abolished the shivering and vasoconstriction which normally follow exposure to cold (Smith and Fay 1939). French workers later discovered that anesthesia could be potentiated by promethazine (phenergan) and later devised the "lytic cocktail" consisting of promethazine, diethazine (diparcol), pentidine and chlorpromazine to block the autonomic nervous system.

The use of autonomic blockade in this instance resulted from the belief that cold-stress overwhelmed the autonomic nervous system and the risk of death was greatly increased when

hypothermia was induced by cooling alone (Laborit 1950, Laborit and Huguenard 1951). More recently British investigators have shown chlorpromazine to be the active ingredient in the "lytic cocktail" as far as the body's normal response to cold is concerned (Dundee et al 1953). These workers have also recognized that the autonomic nervous system might be involved in natural hibernation although they were aware of essential differences between the hypothermic stupor induced in patients by artificial hibernation and the torpidity of natural hibernation in other mammals. A hibernator always maintains some residual temperature control and tends to rouse if the temperature of the immediate environment falls to below the freezing point (Spallanzani 1787, Kayser 1953). Non-hibernators that are artificially cooled to low body temperatures seem to lack this characteristic temperature control (Kayser 1953). This control is also said to be absent in hibernators treated with drugs such as nembutal and then exposed to cold (Kayser 1950, 1953, Lyman and Chatfield 1955).

The technique of artificial hibernation has been of value in human cancer and cardiac surgery since its use slows down metabolism and thereby decreases the risk of procedures which involve the temporary interruption of the blood supply to vital organs.

The physiological changes associated with hibernation in mammals, together with the outstanding theories for the mechanisms involved in the induction and maintenance of dormancy have

been reviewed above. It has been noted also that hibernation has many common characteristics with the syndrome which follows hypofunction of the adrenal cortex.

This investigation attempts to show that changes in the microcirculation and blood chemistry of the hibernating hamster are entirely consistent with the view that functional involution of the adrenal cortex is an important factor in the development of the hibernating state.

II. MATERIALS AND METHODS

General

Hamsters of both sexes, supplied by the Golden Nugget Hamstery at an average weight of 80 grams, were divided into two groups. The experimental and cold control animals were placed in individual cages in a walk-in type refrigerator, which was maintained between six and seven degrees centigrade. The room temperature controls were kept in individual cages in an animal room at room temperature. All animals were allowed free access to food and water. The hamsters which were exposed to cold usually hibernated after two weeks to three months. Cold exposure in the autumn induced hibernation most readily and hibernation occurred least frequently in those animals that were first subjected to the cold environment in late spring or early summer. A seasonal factor seemed to be operative in determining the susceptibility of the hamster to the cold environment. However, the induction of hibernation could be accelerated, even in the summertime, by gradually lowering the temperature of the cold room. Also, hamsters that were initially supplied with a large store of food and a generous amount of shredded paper for building nests hibernated most readily. Once hibernation took place, it was interrupted sporadically for a day or so and then the dormant state was resumed. In this series, continuous hibernation lasted from several days to a month or more. During

the interval of wakefulness, the animals usually ate and voided. The fact that the dormant state in hamsters is interrupted by these periods of awakening and feeding explains why this species can hibernate without first accumulating a large amount of body fat.

Nembutal (pentobarbital sodium), ten milligrams per hundred grams of body weight, injected intraperitoneally, was the anaesthetic used throughout this work. Blood for chemical analysis was obtained by cardiac puncture and digital tip blood was used for estimation of clotting time and hematocrit.

Magnesium determination

The magnesium content of the whole blood was determined for alert hamsters maintained at room temperature, and for hibernating hamsters. The magnesium content of the blood serum was obtained for alert hamsters at room temperature, alert animals kept in the cold room for more than nine days (cold controls), hibernating animals and adrenalectomized animals. The magnesium determinations were made by the microcolorimetric method of Orange and Rhein (1951). In this procedure, the proteins of hemolyzed whole blood or blood serum were precipitated with trichloroacetic acid. Titan yellow was added to the clear filtrate and a red lake was produced by the addition of sodium hydroxide. The intensity of the red color was measured in a colorimeter (Spectronic 20) Bausch and Lomb - at a wave length of 560 millimicrons. Working standards containing 2.03, 3.05 and 4.06

milligrams percent of C.P. magnesium metal were run with each batch of samples. The blanks and standards were prepared in the same manner as the blood samples. The results of the determination in milligrams percent of magnesium were converted into milliequivalents per liter by use of the formula:

$$\frac{\text{milligrams per liter} \times 2}{24.3}$$

Sodium and potassium determination

The sodium and potassium content of the blood serum of normal, alert and hibernating hamsters was determined in a flame photometer (Baird). The procedure used was a modification of that of White (1952). Clear filtrate (0.10 ml) was pipetted into a volumetric flask, standard lithium carbonate solution was added and the flask filled to the mark with distilled water. For potassium determinations, a 25 ml flask containing 1 ml of the standard lithium solution was used, while for sodium determinations, the serum was diluted in a 10 ml volumetric flask which contained 0.40 ml of the standard lithium solution. The results were expressed in milliequivalents per liter of sodium or potassium.

Chloride determination

The chloride content of the blood serum of normal alert and hibernating hamsters was obtained by the method of Schales and Schales (1941). A 0.10 ml sample of clear serum was pipetted

into an Erlenmeyer flask and 0.90 ml of distilled water, 1 drop of 0.005 N Nitric acid and 1 drop of 0.5 percent diphenyl carbazone indicator were added. Each sample was then titrated with 0.0102 N mercuric nitrate, delivered from a precision microburette. A standard 0.1 N potassium chloride solution was read with each batch of samples. The results were expressed in milliequivalents per liter of chloride ion.

Measurement of hemorrhagic tendency

Hibernating, alert cold room control and room temperature control hamsters were tested for hemorrhagic tendency by determining their susceptibility to petechial formation. The methods used were a modification of the moccasin venom test (Arendt and Fulton 1955) and an original positive pressure test. In carrying out the venom test a standard solution of 0.01 percent moccasin (Agkistrodon piscivorus) venom in mammalian Ringer's solution was prepared. The standard solution was kept refrigerated and diluted five times with Ringer's solution for each test. Each determination consisted of the injection of 0.01 cc of diluted venom between the layers of a double membrane preparation of the cheek pouch of a hamster by means of a 1 cc tuberculin syringe fitted with a number 25 needle. Exact petechial counts were made on the injected cheek pouch of each animal at 15 minute intervals for a period of one hour, at a magnification of 27 times. Both control and hibernating animals were given an

anesthetic dose of nembutal prior to testing.

The positive pressure test consisted of the application of a constant pressure of 100 mm Hg to the everted cheek pouch for a period of eight minutes. Petechial counts were made as described in the venom test. The pressure was applied to an area 17 mm in diameter by means of a membrane of dental dam rubber attached to a metal tambour. The membrane was inflated and the pressure measured by a pressure bulb and mercury manometer attached to the pressure device. The positive pressure device is shown in Figure 1.

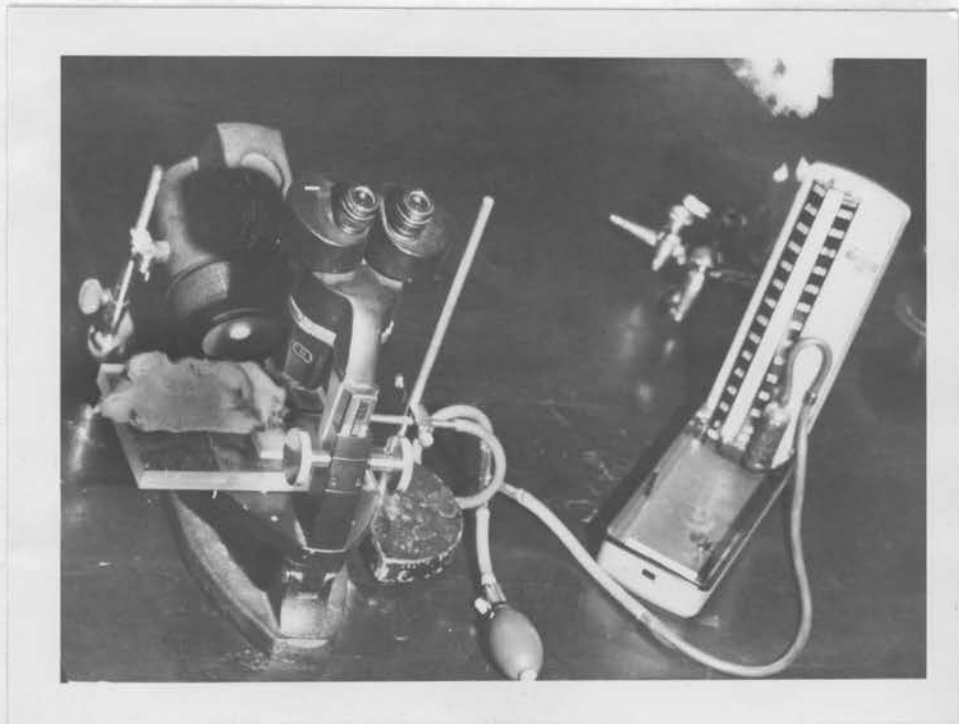


Figure 1. Device for applying positive pressure to cheek pouch

The metal tube clamped to the stand in the center of the photograph is expanded into a hollow disc 17 mm in diameter. A rubber membrane sealed to the disc is in contact with the cheek pouch. The cheek pouch of the anesthetized hamster is pinned to the plastic transilluminating dish shown on the stage of the microscope. The membrane is inflated and the pressure measured by means of the rubber bulb and manometer shown at the right. Petechial counts are made with the aid of the wide-field dissecting microscope.

Hemoconcentration determination

Hemoconcentration was measured from samples of blood obtained from digital tips. The blood was secured by excision of the nail from a digit of the front paw. The blood was drawn into a heparinized capillary tube and one end of the tube was sealed by means of a gas microburner. The tube was then spun for 5 minutes on a high speed centrifuge and read in a micro-hematocrit reader.

Blood clotting time

Blood clotting time was determined upon digital tip blood obtained in the manner described for the hemoconcentration determination. The blood was drawn into a capillary tube and pieces of tube were broken off at intervals. The clotting time was reported as the number of seconds which elapsed from the filling of the tube from a fresh drop of blood, to the appearance of fibrin threads between the broken ends of the tube.

The microcirculation of the cheek pouch

The microcirculation of the hamster cheek pouch was studied during acute and chronic exposure to cold and during hibernation. The everted cheek pouch was pinned to a plastic transilluminating dish according to the procedure developed at the Biological Laboratories of Boston University. Microscopic observations were made at 100x by means of a compound microscope.

Photomicrographs were made at the same magnification. Measurements of the diameters of the blood vessels were carried out with a calibrated eyepiece micrometer. Arterioles and venules between 15 and 30 microns in mean diameter were studied at this magnification. More extensive areas of the cheek pouch were examined at magnifications of from 10x to 70x by means of a wide-field dissecting microscope. All non-hibernating animals received an anesthetizing dose of nembutal prior to the observations.

In order to separate, if possible, the microcirculatory phenomena of hibernation from those due to cold exposure, per se, it was decided to study the microcirculation of the cheek pouch during local cooling of various areas of the body, local cooling of the cheek pouch, acute whole body exposure to cold and chronic whole body exposure to cold, as well as the changes which could be observed during dormancy.

Local cooling of body areas was produced by applying cotton wool, soaked in water at two degrees centigrade, to a hind foot and to a shaved area of the skin of the back. The cheek pouch was cooled by the direct application of cold Ringer's solution (five degrees centigrade). Acute whole body exposure to cold was obtained by enclosing the hamster within a coil of copper tubing and circulating water at five degrees centigrade through the tubing by means of an electrically driven pump. The apparatus is shown in Figure 2. Chronic exposure to cold consisted of placing the animals in a refrigerator kept between six and seven degrees centigrade from 10 to 30 days. These

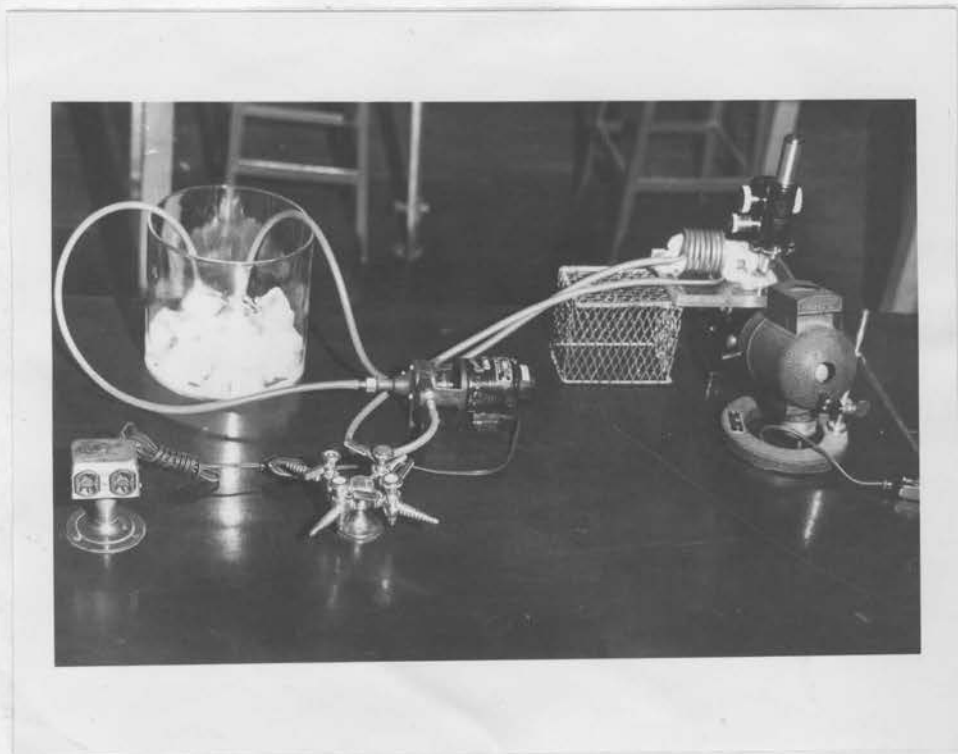


Figure 2. Apparatus for rapid cooling

The electrically driven pump shown in center of photograph circulates ice water from battery jar on left, through copper coil surrounding body of hamster on right. The cheek pouch of the anesthetized hamster is pinned to the plastic transilluminating dish and is observed with the aid of the microscope shown on the right. The wire basket serves to steady the plastic dish and the light source is the lamp in the right foreground.

animals were alert and active until anesthetized.

The hibernating hamsters had entered the dormant state after varying lengths of stay in the refrigerator. When removed from the cold, each hibernant was rolled into a ball, the typical hibernating position, cold to the touch, and had infrequent and almost imperceptible respiratory movements. Cheek pouch temperatures were measured with a Tele-thermometer (Yellow Springs Instrument Company) by inserting the sensing probe of the instrument deep into the cheek pouch contralateral to the one observed under the microscope. Observations upon the cheek pouch microcirculation began within five minutes from the time that the animals were removed from their cages. A double membrane preparation was usually made, in order that circulatory changes might be observed as soon as possible. The hamsters all recovered and appeared to be normal.

Tissue mast cell count

Whole mount preparations of excised cheek pouches of control and hibernating hamsters were made according to the method of Zahl and Nowak (1950) for mouse skin. Each cheek pouch was split longitudinally, stretched over a cardboard frame, fixed in absolute ethanol, and stained with 0.01 percent aqueous methylene blue. The tissue mast cells in each preparation were counted under a compound microscope at a magnification of 150. Counts were made in five different unit areas (0.3 sq.

mm. each) of each preparation with the aid of a whipple disc (Fulton and Maynard 1953). In addition, five high power (645x) fields in each preparation were examined to determine the cell morphology.

Adrenalectomy and adrenal weights

Hamsters were adrenalectomized in a one-stage operation. A dorsal mid-line skin incision about 4 cm. long was made from the tenth thoracic to the third lumbar vertebra. The skin was retracted laterally and a stab wound placed in the muscle wall anterior to the upper pole of each kidney. The adrenals were removed through the stab wounds. The muscle incisions were sutured with silk and the skin incision was closed with skin clips.

The adrenal glands were placed in dry, weighed, stoppered glass vials and weighed on an analytical balance. Both adrenals of a given animal were weighed together.

III. RESULTS

Magnesium in whole blood

The mean level of magnesium in the whole blood of six normal alert hamsters kept at room temperature was 4.06 milligrams percent or 3.34 milliequivalents per liter. Eight hibernating hamsters had somewhat less magnesium in their blood, averaging 3.34 milligrams percent or 2.75 milliequivalents per liter. These results are shown in Table 1. The magnesium level in the whole blood of hamsters has not been reported elsewhere.

Magnesium in blood serum

Both hibernation and adrenal insufficiency were accompanied by an increase in the magnesium content of the blood serum. Eight normal hamsters kept at room temperature had an average of 2.79 milligrams percent or 2.29 milliequivalents per liter of magnesium in their blood serum. Five animals kept in the cold room for from three to five months, but not hibernating, had a very low value for the magnesium content of the serum, namely an average of 2.07 milligrams percent or 1.70 milliequivalents per liter. Eight hibernating hamsters had an average value for the magnesium content of the blood serum of 3.81 milligrams percent or 3.13 milliequivalents per liter.

Table 1.

Magnesium in whole blood in milligrams percent

Normal	Hibernating
3.50	2.60
4.45	3.70
4.45	2.60
4.45	3.50
4.00	3.25
3.50	3.50
----	3.80
----	3.80
Average 4.06 \pm 0.19	3.34 \pm 0.17
t value for difference between means = 2.80	

Nine adrenalectomized hamsters from which blood samples were taken on the fourth day after the operation had an average value of 3.74 milligrams percent or 3.07 milliequivalents per liter for the magnesium content of their serum.

In four cases it was possible to obtain blood from the same animals prior to exposure to cold and during hibernation. These animals had an average of 2.96 milligrams percent or 2.44 milliequivalents per liter of magnesium in their blood serum before exposure to cold and during hibernation the mean level of magnesium had increased to 4.06 milligrams percent or 3.34 milliequivalents per liter.

A rise in the magnesium level of the blood serum has been reported to accompany hibernation in the hedgehog (Suomalainen 1938), the bats Myotis lucifugus and Epetsicus fuscus, the thirteen lined ground squirrel and the golden hamster (Folk et al 1956). Folk's values for the golden hamster were 3.30 milliequivalents per liter for normal animals at room temperature and 4.10 milliequivalents per liter while dormant. The results of adrenalectomy upon the magnesium content of the blood serum of the hamster seem to be absent from the literature, but Conway and Hingerty (1946) have reported that the magnesium value of the plasma of normal rats averaged 2.92 milliequivalents per liter while adrenalectomized rats showed a mean level of magnesium in plasma of 4.06 milliequivalents per liter. The magnesium values for blood serum are shown in Tables 2 and 3.

Table 2.

Magnesium in blood serum in milligrams per 100 milliliters

Normal	Cold Controls	Hibernating	Adrenalectomized
3.25	1.98	3.65	4.06
3.25	2.27	3.26	3.60
3.25	2.13	3.65	4.85
2.70	1.98	4.06	4.06
3.26	1.98	4.46	3.38
2.54		3.25	3.38
2.03		4.06	3.25
2.03		4.06	3.05
			4.06
Ave. 2.79 \pm .20	2.07 \pm .07	3.81 \pm .15	3.74 \pm .19
t value for difference between means of normal and hibernating 5.9			

Table 3.

Magnesium in blood serum in milligrams
per 100 milliliters

Animal	Before hibernation	During hibernation
1	3.38	4.06
2	3.38	4.06
3	2.54	4.46
4	2.54	3.65
Average	2.96	4.06

Sodium and potassium in blood serum

Hibernation was accompanied by an increase in the mean level of sodium in the blood serum and not much change in the level of potassium. The average concentration of sodium in the serum of six normal hamsters kept at room temperature was 137.39 milliequivalents per liter and the average level of potassium was 6.05 milliequivalents per liter. These values are very close to the ones for normal hamsters reported by Fulton and Sudak (1954) of 135.0 milliequivalents per liter for sodium and 6.5 milliequivalents per liter for potassium. Snyder and Wyman (1951) found somewhat higher levels in their normal hamsters, namely 169.0 milliequivalents per liter for sodium and 8.9 for potassium.

Eleven hibernating hamsters had a mean level of sodium of 154.26 milliequivalents per liter, while the mean level of potassium, 6.41 milliequivalents per liter, approximated that of the normal animals.

Adrenalectomy and hibernation appear to differ in their influence upon the levels of sodium and potassium in the blood serum of the hamster. Snyder and Wyman (1951) have shown that the adrenalectomized hamster concentrates potassium while the level of sodium remains essentially unchanged. Their values for adrenalectomized hamsters on the fourth postoperative day were 150 milliequivalents per liter for sodium and 18 milliequivalents per liter for potassium. Moreover, these authors

stated that while injections of desoxycorticosterone acetate maintained life in their adrenalectomized hamsters, it did not affect potassium retention. The sodium and potassium values for blood serum are shown in Table 4.

Chlorides in blood serum

Hibernating hamsters showed a slight increase in the amount of chloride ion in their blood serum, as compared to normal animals. Six normal animals, kept at room temperature had an average of 93.1 milliequivalents per liter of chloride while the blood serum of six hibernating hamsters contained a mean of 98.5 milliequivalents of chloride per liter.

Both Addison's disease and experimental adrenal insufficiency are characterized by a drop in the amount of chloride ion in the blood serum while the mineralo-corticoids generally promote urinary retention of sodium and chloride (Turner 1955). Chloride values are given in Table 5.

Hemorrhagic tendency

The hibernating hamster develops petechial hemorrhages much more readily than alert normal hamsters kept at room temperature or those exposed to a temperature of about five degrees centigrade for more than five days. Comparable results were obtained with an original positive pressure test and a modified moccasin venom test.

Table 4.

Sodium and potassium in blood serum

Normals	Sodium meq/liter	Potassium meq/liter
1	128.64	7.02
2	137.09	4.50
3	139.10	7.50
4.	139.00	7.02
5	136.25	5.12
6	144.25	5.13
Average	137.39 \pm 2.23	6.05 \pm 0.52
Hibernating		
1	146.75	6.92
2	144.25	5.58
3	154.25	5.34
4	145.25	5.40
5	161.25	8.87
6	143.13	7.19
7	144.19	5.53
8	142.75	5.03
9	178.50	5.00
10	158.25	6.24
11	178.25	9.38
Average	154.26 \pm 4.07	6.41 \pm 0.45
t value for difference between means for sodium = 2.94		
t value for difference between means for potassium = 0.5		

Table 5

Chloride in blood serum in milliequivalents per liter

Normals	Hibernating
94	98
96	99
97	102
93	99
90	99
89	94
Average 93.3 ± 1.30	98.5 ± 1.10
t value for difference between the means = 4.4	

In the moccasin venom test, the average number of petechial hemorrhages counted during the first hour after the application of diluted venom to the cheek pouch was 3.0 for eight room temperature animals, 2.66 for six cold room animals and 53.33 for six hibernating hamsters.

The results of the positive pressure test were similar. Within the hour after a positive pressure of 100 millimeters of mercury was applied to the pouch for eight minutes, six room temperature animals had an average of 1.80 petechiae while ten hibernating animals had an average of 34.5 petechiae. The petechial hemorrhages appeared at venous junctions with smaller venules and were not observed on arterioles or capillaries. Increased susceptibility to formation of petechial hemorrhages of blood vessels in the cheek pouch of the hamster follows the injection of heparin (Fulton et al 1953) and exposure to X and beta irradiation (Fulton et al 1956). Heparin is an anticoagulant and ionizing radiations are alleged to free anticoagulants into the circulating blood, therefore, it is possible that a circulating anticoagulant is responsible for the absence of thrombosis and susceptibility to petechial hemorrhage in the blood vessels of hibernating animals. Increased "capillary fragility" is also characteristic of hypofunction of the adrenal cortex (Ingle, 1944, Turner 1955). Petechial counts are recorded in Tables 6 and 7.

Table 6

Petechial count 1 hour after application of moccasin venom

Normal	Cold stressed	Hibernating
3	0	45
2	0	42
1	0	53
2	9	65
1	2	70
12	5	45
1	--	--
2	--	--
Average 3 ± 1.3	2.66 ± 1.2	53.33 ± 4.8
t value between means of normal and hibernating animals 11.4		

Table 7

Positive Pressure Test

Petechiae 1 hour after application of pressure	
Normal	Hibernating
2	30
2	50
1	55
2	32
0	14
4	45
	26
	55
	20
	18
Average $1.8 \pm .54$	34.5 ± 4.1
t value for difference between means = 6.6	

Hemoconcentration

Hemoconcentration as indicated by increase in the hematocrit, was a constant finding in hibernating hamsters. Average hematocrit reading for eight normal hamsters kept at room temperature was 52.35 while that for five cold controls was 55.4. Eight hibernating hamsters had an average hematocrit of 63.37. Svihla and Bowman (1952) and McBirnie et al (1953) have reported an increase in the hematocrit in hibernating ground squirrels and hedgehogs respectively. Hemoconcentration is a frequent result of adrenal cortical insufficiency (Ingle 1944). Hematocrit readings are shown in Table 8.

Blood Clotting Time

Hibernating hamsters showed a longer blood clotting time than normal animals kept at room temperature or alert animals exposed to cold. The average blood clotting times were 31 seconds for six room temperature animals, 150 seconds for three cold controls and 240 seconds for six hibernating hamsters. The capillary tube method used with finger tip blood gave much more satisfactory results in our hands than the Lee-White technique and cardiac blood. The values reported here are considerably less than those of Svihla et al (1952). These authors used the Lee-White method on blood obtained by cardiac puncture and they found the average blood clotting time of eleven normal hamsters to be 292 seconds and that for four dormant hamsters

Table 8

Hematocrit readings

Normal	Cold Stressed	Hibernating
53.63	55.00	60.50
51.88	56.00	64.50
57.60	56.00	61.50
55.50	55.50	63.50
50.15	54.50	64.50
48.25		62.50
48.50		64.00
53.25		66.00
Ave. 53.25 ± 1.16	55.40	$63.37 \pm .63$
t value between means of normal and hibernating = 9.0		

in excess of fifty minutes. Blood clotting times are shown in Table 9.

Microcirculation of the cheek pouch

Local cold The effects of local cooling of the hind foot, the shaved skin of the back and the cheek pouch were arteriolar dilation accompanied by slow, chunky flow. In the eight animals tested, this result was seen within five minutes of the application of the cold stimulus. In no case was the application of the cold stimulus to any of the parts mentioned followed by reflex vasoconstriction of the blood vessels of the cheek pouch.

Sudden cooling of the whole body Six hamsters were rapidly cooled to a deep cheek pouch temperature of between 25 and 27 degrees centigrade in the apparatus described above. The average time required to produce a 10-degree centigrade fall in cheek pouch temperature was three minutes. The blood vessels of the cheek pouch were observed continuously prior to and during the cooling process and there was no reflex vasoconstriction noticed. The first effect observed was a slight dilation of smaller arterioles between fifteen and thirty microns in diameter. In some cases, the initial dilation was followed by a slight constriction, possibly a return to original size. The initial dilation was seen within three minutes of the start of cooling, while the constriction or return to size followed five to ten minutes later. The final effect,

Table 9

Blood clotting time in seconds

Normal	Cold stressed	Hibernating
30	150	270
35	180	240
30	120	300
30		270
30		180
30		180
Ave. $31 \pm .83$	150	240 ± 22.8
t value for difference between means of normal and hibernating = 12.3		

in every case, was a dilation which persisted until the pump was shut off and the rewarming process was well advanced. The arteriolar dilation extended to smaller arterioles and resulted in increased numbers of capillaries opening up and flowing. The neighboring venules usually reacted in the same way as the arterioles, but to a lesser degree.

Chronic exposure to cold Six alert hamsters were exposed to cold in the refrigerator for ten days and were then studied to determine the effects of chronic exposure to cold upon the microcirculation of the cheek pouch. The deep cheek pouch temperatures ranged from 26 degrees centigrade to 33 degrees centigrade, and averaged 28.3 degrees centigrade. These animals were injected with an anesthetizing dose of nembutal while they were in the refrigerator and left there until they were used. The cheek pouch temperatures were proportional to their length of stay in the refrigerator after injection of nembutal. Nembutal anesthesia seems to inhibit the heat producing mechanism since unanesthetized hamsters maintain an essentially normal body temperature under the same conditions of chronic exposure to cold until just prior to hibernating.

Chronic exposure to cold did not produce any dramatic changes in the microcirculation of the cheek pouch. On occasion, the rewarming of these animals was accompanied by a slight arteriolar constriction and slow or reversed flow of blood in these vessels. However, packing of the red cells, and stagnant or static flow were not seen in the blood vessels of these animals.

Hibernation The blood vessels of the cheek pouches of hibernating hamsters presented an entirely different appearance from those of animals subjected to various forms of cold stress. While various types of cold stress per se appeared to have little effect upon the microcirculation of the cheek pouch, hibernation produced profound changes, similar in some respects to those described by Wyman et al (1953) for hamsters suffering from experimental adrenal insufficiency. A total of twenty-five hibernating hamsters was studied in this phase of the investigation and a composite picture of the results is presented. The handling of the animals consequent to everting and pinning their cheek pouches to the transilluminating dish, together with the warmth of the laboratory, initiated the arousal process in the hibernating animals. However, the microcirculation could be observed for at least 40 minutes before the struggles of the animals halted the procedure.

The everted cheek pouch of the dormant animal was very red proximally, but quite pale distally, when first observed. This suggested that the periphery of the pouch had a minimal circulation during dormancy and microscopic observations confirmed the suggestion. The deep cheek pouch temperature averaged 12 degrees centigrade in the first five minutes after removal of the animals from the cold room and at this time many of the largest arteries and veins of the pouch were tightly packed with red cells. These vessels were completely stagnant and no blood flowed through them. At this time, arterial

The following table shows the results of the experiment. The first column is the number of trials, the second column is the number of correct responses, and the third column is the percentage of correct responses.

Number of trials	Number of correct responses	Percentage of correct responses
10	7	70%
20	14	70%
30	21	70%
40	28	70%
50	35	70%
60	42	70%
70	49	70%
80	56	70%
90	63	70%
100	70	70%

Figures 3, 4 and 5 show an area of the cheek pouch of a hibernating hamster photographed at 6.5, 13 and 20 minutes respectively, from the time that the animal was removed from the cold room. Magnification at the microscope is 10x. The Y-shaped vessel in the center of the figures shows a progressive narrowing as the flow increases. Other vessels show the alternate dilations and constrictions of "slow" vasomotion.

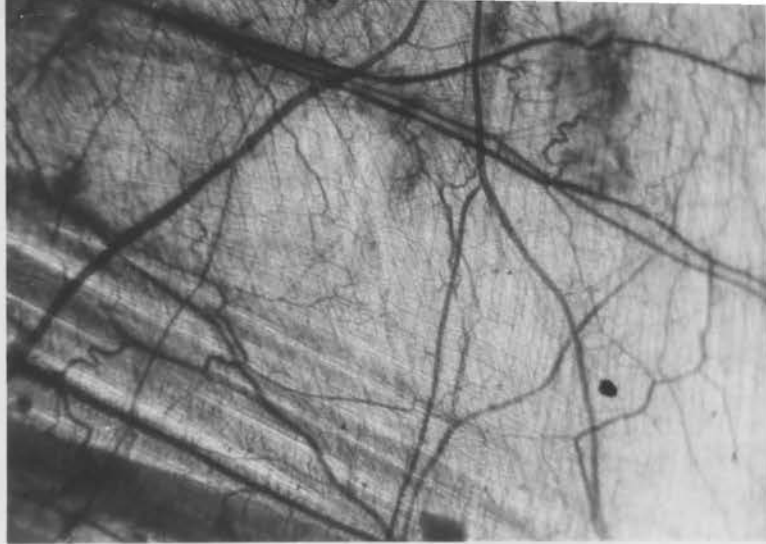


Figure 3.

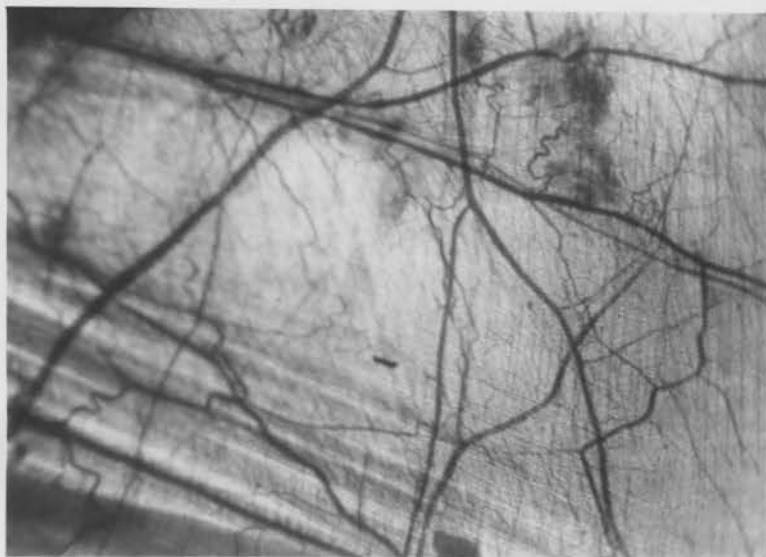


Figure 4.

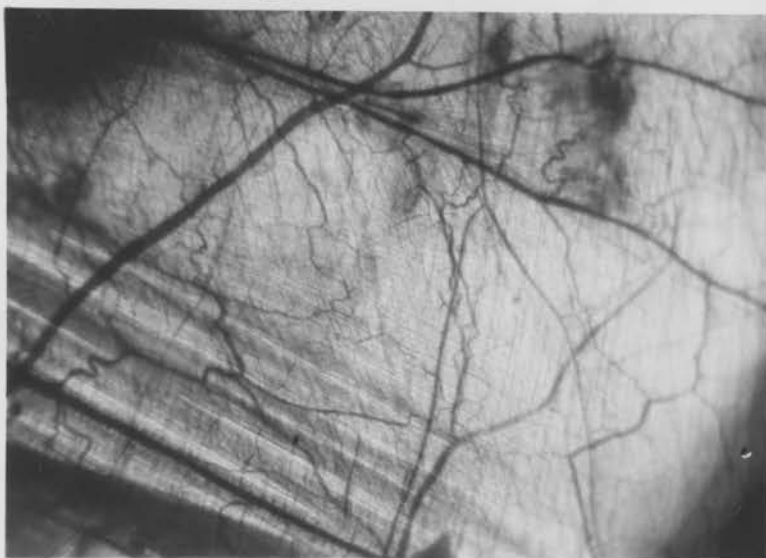


Figure 5.

blood was supplied to the pouch largely by way of arteries with a mean diameter of less than 50 microns and the venous return was by way of the smaller veins. Thus, the microcirculation of the cheek pouch as observed in the very early stage of the arousal process was characterized by a very limited flow of blood which was confined for the most part to smaller arteries and veins. The principal pathway which the blood followed was by way of smaller arteries from the proximal end of the pouch, along its length, to the distal end. At the distal end of the pouch much of the blood flowed into circumferential vessels which resembled the arcuate arteries described by Nicoll and Webb (1955). The circumferential vessels conveyed the blood along the periphery of the cheek pouch and up to its proximal end. The blood supply to the cheek pouch at this time was furnished mostly by twigs from the circumferential vessels. Reversed flow occurred frequently in the arteries which supplied the circumferential vessels. At times, the direction of flow was reversed in one end of an artery while the other end continued to flow in the original direction, and the flow from the two directions would meet and pass into an arterial twig at right angle to the plane of the surface. The "Indian Club" dilations mentioned by Nicoll and Webb (1955), were not seen.

In the interval from five to fifteen minutes after removal from the cold room, the cheek pouch temperature increased

to about 16 degrees centigrade. At this time, the velocity of the blood flow increased in the patent larger vessels and numerous arterioles, capillaries and venules filled with blood and commenced to flow so that distribution of blood to the tissues was greatly improved.

During the next fifteen minutes, the deep cheek pouch temperature reached 20 degrees centigrade. At this time, the packed red cells in the larger, previously static, blood vessels separated and blood flow started, first in an oscillatory to-and-fro movement and later in an intermittent chunky flow. Some of the larger arterioles showed a beaded appearance at this time. The beaded appearance was due to alternate dilations and constrictions along the lengths of the arterioles. The flow characteristics soon changed from intermittent to smooth, regular high velocity flow. Between thirty and forty minutes from the time of removal of the animal from the refrigerator, the cheek pouch temperature rose to above 23 degrees centigrade and a good blood flow throughout the pouch was fairly well established. However, some small vessels were still static and others flowed only intermittently.

Soon after the cheek pouch temperature reached 21 degrees, the wakening hamster struggled to right itself and observations upon the cheek pouch became very difficult. The righting reflex never took place at temperatures below 21 degrees centigrade and it appeared that this was a critical temperature for

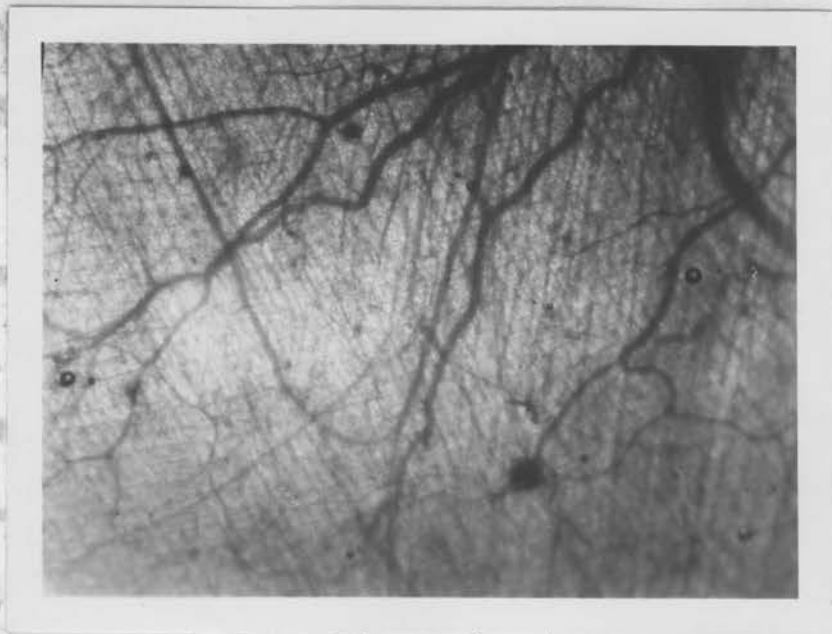


Figure 6.

Area of cheek pouch of hibernating hamster 9 minutes after removal from cold room. Small vessels at bottom of photomicrograph are starting to flow. Magnification 30x at microscope.

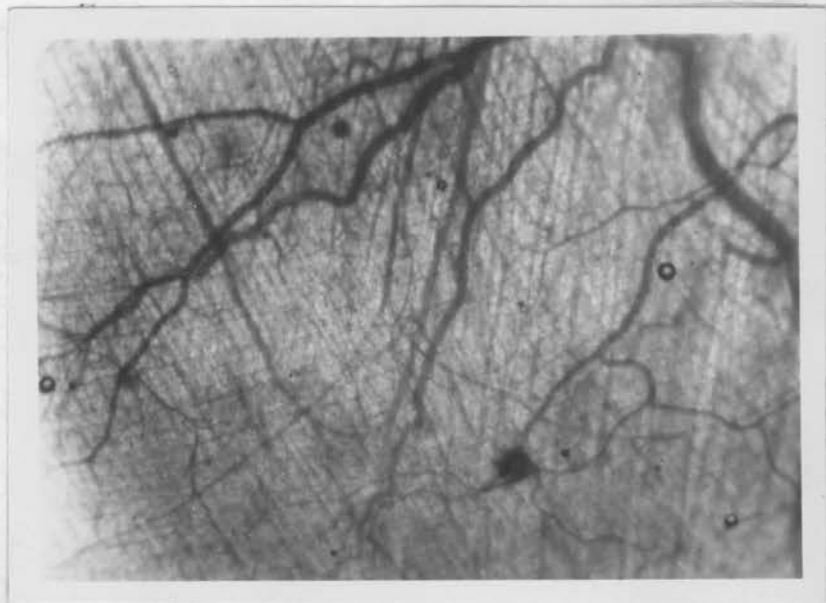


Figure 7.

Small area as in Figure 6 taken at 17 minutes after removal of animal from cold room. Increased blood flow and vascularity and narrowing of some of the vessels. Magnification 30x at microscope.

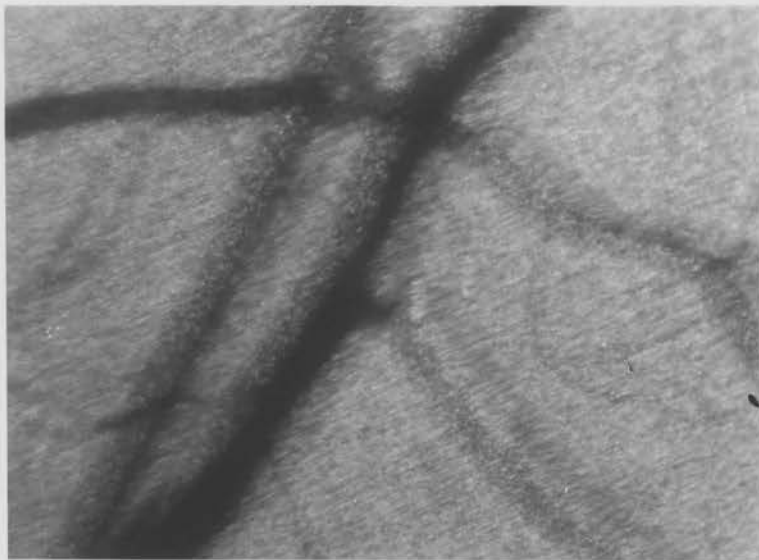


Figure 8.

Area of cheek pouch of hibernating hamster showing start of blood flow in artery (left) and vein (right) 5.5 minutes after removal of animal from cold room. Magnification at microscope 100x.

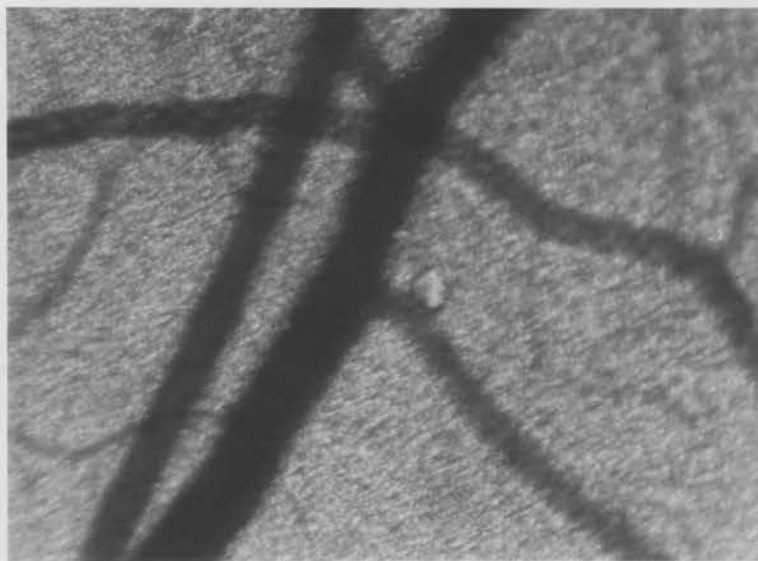


Figure 9.

Same area as in Figure 8 taken 11 minutes after removal of animal from cold room. Flow has been established in previously empty vessels. Magnification 100x at microscope.

completion of the righting reflex in this species.

Although there is marked stasis and decrease in blood flow in the vessels of the cheek pouch of the hibernating hamster, this stasis was not correlated with arterial vasoconstriction characteristic of adrenalectomized animals (Wyman et al 1953), nor was the resolution of the stasis accompanied by dilation of the vessels involved. Instead, the walls of the static vessels seemed to be atonic and stretched by the packed corpuscles within. When flow started, the previously dilated vessels narrowed and their walls appeared to be more distinct. "Rapid" vasomotion was absent from the cheek pouch vessels of the hibernating hamster and the alternate dilations and constrictions along arterioles were minutes in duration and similar to the slow type of vasomotion described by Chambers and Zweifach (1944). Perhaps stasis in hibernation is a result of the extreme bradycardia and hemoconcentration characteristic of dormancy. Loss of tone of vascular smooth muscle may be a contributing factor, but this last possibility has not been tested experimentally. Cold stress per se failed to produce the vascular changes observed in hibernating animals. Possibly the stasis and slow flow of hibernation are adaptations which conserve blood for vital areas. The cheek pouch is known to rewarm early in the arousal process (Lyman and Chatfield 1950) and this accounts for the resolution of the stasis which has been described above. The change in cheek pouch temperature during arousal is shown in Table 10.

Table 10

Deep cheek pouch temperature during arousal in degrees centigrade. Average of 10 experiments.

Elapsed time in minutes	Temperature
0	10.0
5	12.6
10	13.9
15	15.6
20	16.9
25	18.2
30	19.8
35	22.1

Cheek pouch mast cells

There were approximately equal numbers of tissue mast cells in the cheek pouches of hibernating and normal hamsters and the distribution of mast cells did not differ significantly in the pouches of the two groups. Cold stress is reported to result in a decrease of these cells in the skin of the rat ear and an increase in the skin of the abdomen (LeBlanc and Rosenberg 1957). Cortisone treatment tends to reduce the number of mast cells in the hamster cheek pouch (Fulton and Maynard 1953). Apparently hibernation is not accompanied by factors which influence the mast cells of the cheek pouch although mast cells in other sites may be affected. Mast cell counts are given in Table 11.

Adrenal weights

There was no significant difference between the adrenal weights of normal and hibernating hamsters. This confirms the findings of Deane and Lyman (1954). However, there is histochemical evidence for increased activity of the zona glomerulosa and hypofunction of the zona fasciculata of the adrenal cortex of hibernating hamsters (Deane and Lyman 1954, Demers 1959). During removal of the adrenal glands, the viscera of hibernating hamsters appeared to be very hyperemic. Visceral hyperemia is characteristic of animals suffering from adrenal cortical deficiency (Turner 1955). Adrenal weights are listed in Table 12.

Table 11

Number of mast cells per 5 unit areas of the
cheek pouch

Normal	Hibernating
373	464
416	607
562	471
395	392
528	422
	457
Ave. 455 \pm 38	469 \pm 30
t value for difference between means = 0.41	

Table 12

Weight of both adrenals compared to total body weight

Normal		Cold controls		Hibernating	
glands	body	glands	body	glands	body
0.019gm	100gm	0.015gm	120gm	0.022gm	100gm
0.014	94	0.021	114	0.023	96
0.008	100	0.026	106	0.014	92
0.012	100	0.019	120	0.028	100
0.017	92	0.024	112	0.015	85
---	--	---	--	0.018	100
---	--	---	--	0.011	100
Av. 0.014 ± .002	97	0.021 ± .002	114.4	0.019 ± .002	96.1

In summary, this investigation has established that hibernation in the golden hamster is accompanied by alterations in the electrolyte balance and in the microcirculation of the cheek pouch. Hibernating animals showed a slight decrease in the level of magnesium in the whole blood and significant increases in the concentrations of sodium, magnesium and chloride in the blood serum. However, the potassium values of the serum of dormant animals did not differ significantly from those of the controls. Microcirculatory phenomena characteristic of hibernation include stasis, erythrocyte packing and slow flow, but a generalized vasoconstriction was not seen. Furthermore, it has been shown that cold stress, by itself, failed to produce these changes in the microcirculation of the cheek pouch.

"Slow" vasomotion was noted during the arousal process. The blood of hibernating hamsters was concentrated and the clotting time was increased. A hemorrhagic tendency in hibernating animals was evidenced by increased susceptibility to the formation of petechial hemorrhages upon the application of moccasin venom or positive pressure to the cheek pouch. Finally, the weight and gross anatomy of the adrenal gland as well as the number and distribution of the tissue mast cells of the cheek pouch have been shown to remain within normal limits during hibernation.

Lengthened clotting time of the blood and absence of gross morphological changes in the adrenal glands of hibernating

hamsters have been reported previously by others (Svihla et al 1952), (Deane and Lyman 1954), and during the progress of the present work, other evidence for the increase in the level of magnesium in the blood serum of hibernating hamsters has appeared (Folk et al 1956).

IV. DISCUSSION

The physiology of hibernation has been of interest to biologists for hundreds of years and has engaged the attention of Jenner, Spallanzani and Harvey Cushing among others. Nevertheless, in spite of the many parameters of the hibernating state that have been explored, the mechanism by which an active warm-blooded mammal can in a relatively short time, reverse its evolutionary history and revert to the poikilothermy of its ancestors, has not been explained satisfactorily.

Many students of hibernation have attributed the induction of dormancy to an altered endocrine balance set in motion by exposure to cold, but this hypothesis has been based largely upon the study of a few animals taken in the field and the evidence for it is not conclusive (Kayser 1950).

This investigation has shown that a number of physical and chemical changes in the circulation of the blood in hibernating hamsters are similar to those seen in adrenalectomized laboratory mammals (Ingle 1944, Snyder and Wyman 1951, Wyman et al 1953) and Addisonian patients (Turner 1955). However, there are important differences between the states of adrenal insufficiency and dormancy, and hibernation is not induced by simple hypofunction of the adrenal cortex.

The adrenal gland of the hamster has been described by Knigge (1954), Deane and Lyman (1954), Chester Jones (1957). It is about the size of a small pea bean and weighs about eleven

milligrams in the male and slightly less in the female, contrary to the condition in most mammals where the female gland usually is heavier. The gland is surrounded by a connective tissue capsule which encloses a cortex and medulla. The cortex contains the usual three zones and histochemical tests indicate that it is relatively free of lipid. The hamster, some other hibernants and some ruminants apparently secrete adrenocortical steroids without the occurrence of lipid droplets (Chester Jones 1957).

The pattern of cortical secretion in the hamster adrenal has been recently revealed by chromatographic analysis of the effluent from the adrenal vein and it appears that the principal steroid is hydrocortisone (compound F) which is secreted at a rate of 0.5 milligrams per kilogram of body weight per day. This rate of secretion is from three to about fifty times smaller than that found in other common mammals (Schindler and Knigge 1959). Aldosterone was not detected in the secretion of the hamster's adrenal but this is not surprising since this hormone is usually secreted in very low concentration and may have escaped detection.

The secretion of compound F appears to be controlled normally by ACTH (Chester Jones 1957) while the factors responsible for the regulation of the output of aldosterone are not known. Increased aldosterone appears in the adrenal venous blood of dogs or rats following reduced sodium intake or ACTH administration. Farrell (1958) has postulated that a center located in the caudal diencephalon or anterior midbrain secretes a humoral tropic factor, or that possibly delta corticotropin from the anterior pituitary stimulates aldosterone secretion by the zona glomerulosa, and that the center which

controls aldosterone secretion is influenced by impulses which arise in other parts of the brain stem and in peripheral receptors.

An animal completely deprived of adrenal cortical steroids suffers excessive loss of sodium by way of the kidneys. Chloride is lost, also, but not as rapidly as sodium, and the level of these ions in the plasma falls. At the same time, the kidney is unable to excrete potassium adequately and the potassium concentration of the plasma rises. On the other hand, sodium retention can be brought about by aldosterone and to a lesser extent, by other cortical steroids. The marked sodium retention which often occurs after ACTH administration and which is often seen clinically, is probably due to increased secretion of aldosterone (Chester Jones 1957) and the rise in the sodium level of the plasma of the hibernating hamster may result from a slight increase in aldosterone or other mineralocorticoid secretion during dormancy. The same mechanism may account for the increase in chloride ion concentration in the plasma during hibernation.

The influence of adrenal corticosteroids upon the movement of the potassium ion is not well understood, but hydrocortisone is said to be a more potent stimulus to potassium excretion than aldosterone or other mineralocorticoids (Leaf 1960), and the pronounced hyperpotassemia of the adrenalectomized hamster may be caused by its inability to excrete this ion efficiently in the absence of hydrocortisone. The potassium value of the blood of the hibernating hamster was found to remain within normal limits.

The magnesium level of the plasma was found to rise in both adrenal insufficiency and hibernation. There are no experi-

mental data available to explain the relation of adrenal corticosteroids to movements of magnesium in the plasma. One might speculate that the excretion of magnesium depends upon the ratio of hydrocortisone to aldosterone so that either increased aldosterone or decreased hydrocortisone would result in a shift of this ion into the plasma.

Hemoconcentration also is common to both adrenal insufficiency and hibernation. Many types of stress will bring about disturbances in water balance and both pituitary and adrenal cortical hormones are involved in homeostasis of water in the body. However, it is known that aldosterone not only causes sodium retention but also brings about a shift of water from the blood into the tissues (Leaf 1960) and this may contribute to the hemoconcentration of hibernation, but not of adrenal insufficiency.

The microcirculation of the cheek pouch of cold stressed and hibernating hamsters showed stasis and slow flow. The stasis was much more extensive in the hibernating animals and was accompanied by erythrocyte packing. However, despite the stasis and slow flow, intravascular clotting did not take place. In addition, neither the cold stressed nor hibernating hamsters had any evidence of generalized vasoconstriction in their blood vessels. Stasis and slow flow without an accompanying vasoconstriction have been reported by others. Lynch and Adolph (1957) produced local cooling in the cheek pouch of the hamster by means of a stream of cold air or by irrigating the pouch with cool Locke's solution, and they also cooled the whole body by packing an animal in ice until

its colonic temperature fell to between eleven and five degrees centigrade. No anesthetic was used. Their findings were that local cooling of the cheek pouch produced stasis in some capillaries and venules, but not in arterioles while whole body cooling was followed by stasis in arterioles as well. They also noted that stoppages of blood flow in the capillaries increased greatly in number as the temperature decreased, and the blood flow returned gradually to all vessels during the process of rewarming. The average caliber of the blood vessels did not change markedly and the slow flow was attributed to the effect of cooling upon the cardiac rate and upon the physical characteristics of the blood.

More recently, Wyman and Drapeau (1959) have studied the vascular reactions to cold in the cheek pouch of the intact and in the adrenalectomized hamster. Their animals were lightly anesthetized with ether and exposed to cold for four hours in a chamber maintained at two degrees centigrade. The final body temperature of the animals was about seventeen degrees centigrade. The cold stressed intact hamsters showed a progressive vasoconstriction which had a latent period of about thirty minutes. The long latent period implies humoral rather than nervous mediation of the vasoconstriction and this supposition is strengthened by the fact that adrenalectomy impaired the vasoconstrictor response to cold and the vasoconstrictor reaction was restored in cortisone treated cold stressed hamsters.

The reactions of the microcirculation of the cheek pouch of the hamster to cold stress seem to vary with the anesthetic used, the rate of cooling and the final temperature of the animal.

However, the changes in the microcirculation of the cheek pouch of the cold stressed and hibernating animals observed in the course of this investigation seemed to follow the pattern described by Lynch and Adolph (1957). On the other hand, the fact that adrenalectomy interferes with the development of vasoconstriction in response to cold (Wyman and Drapeau 1959) is consistent with the view that the hibernating hamster is deficient in the particular adrenal corticoid essential for vasoconstriction in response to hypothermia.

Increased blood clotting time and absence of intravascular clotting of the blood of the hibernating hamster may result from the presence of an intravascular anticoagulant which, if present, would also explain the increased susceptibility to petechial formation in these animals. A possible source of this anticoagulant would be the mast cell which is known to produce heparin (Fulton et al 1957). Examination of the mast cells of the cheek pouch of the hibernating hamsters showed them to be about the same as those of normal animals in number distribution and morphology. However, mast cells are present in other sites in the hamster and the possibility remains that hypothermia stimulates the release of an anticoagulant from these cells.

Finally, the results of this work prove that specific physical and chemical changes in the circulation of the blood are associated with hibernation in the golden hamster. Moreover, these changes are in those aspects of the circulation which are considered to be regulated by hormones of the adrenal cortex

(Jones 1957). Differences observed above between the hibernating and adrenalectomized animals may well depend on the complete absence of adrenal cortical steroids from adrenalectomized animals as contrasted with either decline or altered ratios in the production of the adrenal cortical hormones during the induction and maintenance of dormancy. Arrangements have been made to pursue the theory that the cortical steroids play an important role in hibernation by making assays of these hormones in hibernating and normal animals.

V. SUMMARY

1. The whole blood of hibernating hamsters contains less magnesium than the blood of normal animals kept at room temperature.
2. The blood serum of hibernating hamsters contains more magnesium than the serum of normal hamsters or that of hamsters acclimated to cold.
3. The blood serum of adrenalectomized hamsters contains about the same amount of magnesium as the serum of hibernating hamsters.
4. The mean levels of sodium and chloride are higher in the blood serum of hibernating hamsters than in the serum of normal animals kept at room temperature.
5. The hibernating hamster has a longer blood clotting time, a greater hemorrhagic tendency and more hemoconcentration than normal animals or those acclimated to cold.
6. The microcirculation of the cheek pouch of hibernating hamsters is characterized by atonic and dilated blood vessels, stasis, erythrocyte packing, slow and reversed blood flow and "slow" vasomotion and cold stress per se does not account for these phenomena.
7. The number, morphology and distribution of the tissue mast cells of the cheek pouch are essentially the same in hibernating and normal animals and hibernation is without significant effect upon the weight and gross morphology of the adrenal gland in the hamster.

8. Mineral metabolism, the state of small blood vessels and the characteristics of the blood flow are known to be influenced by variation in output of cortical steroids by the adrenal glands and the fact that these parameters change during dormancy suggests that the adrenal cortex has an important role in the induction and maintenance of the hibernating state in the golden hamster.

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ABSTRACT

MINERAL METABOLISM AND MICROCIRCULATION IN THE HIBERNATING HAMSTER (Library of Congress No. Mic. 61)

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Boston University Graduate School, 1961

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Mineral metabolism and certain circulatory phenomena, all of which are thought to be influenced by the steroid hormones of the adrenal cortex, were studied in hibernating, cold stressed, and normal hamsters.

Hibernation was induced by prolonged exposure to a temperature of 6°C. Chronic cold stress resulted from exposure of hamsters to the same conditions which induced hibernation, but for a shorter time. Acute cold stress was brought about by surrounding the body of the test animals with a copper coil through which ice water was pumped. Body temperature was measured by means of a thermistor type thermometer with the sensing probe inserted deep into the cheek pouch.

Blood was withdrawn by cardiac puncture and the serum was analysed for its content of sodium, potassium, magnesium, and chloride. Determinations of sodium and potassium were made by flame photometry (White, J.U., *Analyt. Chem.* 24: 394-399, 1952). Determination of magnesium was made by a microcolorimetric method (Orange, M., and H. C. Rhein, *J. Biol. Chem.* 189: 379-386, 1951). The chloride content of the serum was obtained by titration of the blood serum with mercuric nitrate delivered from a microburette (Schales, O., and S. S. Schales, *J. Biol. Chem.* 149: 879-884, 1941).

The circulatory phenomena studied include direct microscopic observation of the microcirculation of the cheek pouch at magnifications from 10x to 100x, the susceptibility of the blood vessels of the cheek pouch to formation of petechial hemorrhages, hemoconcentration and blood clotting time. The tissue mast cells of the cheek pouch were also studied to determine if changes in the number and morphology of these unicellular endocrines could be correlated with the fact that thromboses do not form in the blood vessels of hibernating animals in spite of their sluggish circulations.

Susceptibility to formation of petechiae was evaluated by an original positive pressure method and by the use of the moccasin venom test (Arendt, K. A., and G. P. Fulton, A. J. Physiol. 183: 594, 1955). Hemoconcentration and blood clotting time were determined upon digital tip blood by capillary tube methods. The tissue mast cells were studied by a sampling procedure (Fulton, G. P. and P. L. Maynard, Proc. Soc. Exp. Biol. Med. 84: 259-260, 1953).

The results of this investigation show a significant rise in the levels of sodium, magnesium, and chloride ions in the blood serum of hibernating hamsters, whereas the value for potassium remains essentially normal.

The microcirculation of the cheek pouch of hibernating hamsters is characterized by dilated blood vessels, stasis, erythrocyte packing, slow and reversed flow and "slow" vasomotion. Cold stress per se does not account for these changes. Moreover, the blood

vessels of the cheek pouch in hibernating hamsters, are much more susceptible to the formation of petechial hemorrhages than those of cold stressed or normal animals. Hemoconcentration and significantly lengthened blood clotting time were constant findings in the hibernating animals.

The numbers and morphology of the tissue mast cells were not influenced by the transition to the dormant state in these animals and the absence of thrombi from the circulation of hibernating hamsters is apparently not due to a circulating anticoagulant released by mast cells of the cheek pouch.

The fact that the chemical and physical parameters of the circulation described above are influenced by hormones of the adrenal cortex suggests that altered ratios of adrenal steroid production may influence the induction and maintenance of dormancy in this species.

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