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Some Effects of Cold-Acclimation on the Biochemistry and Histology of the Hamster Kidney.* (28294)

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The striking enlargement of the kidney which accompanies cold-acclimation in the hamster, *Mesocricetus auratus*(1), suggests that this organ may play a particularly important compensatory role in maintenance of thermal homeostasis. Since growth reflects cellular changes, a basic understanding of the nature of this renal enlargement must await considerable histological, cytological, and biochemical information. Studies on this subject have not been reported by other workers, and we have reported our results only briefly (2,3). This communication documents these preliminary notes, and presents in detail other studies not previously reported. It includes (a) a histological analysis of the early and advanced stages of acclimation, (b) assays of total renal DNA, protein, and microsomal nitrogen, (c) assays of microsomal DPNH-cytochrome c reductase, and (d) assays of succinoxidase, succinic dehydrogenase, and transaminase of kidney homogenates. Body and kidney weights of both heat- and cold-acclimated male hamsters, and of cold-acclimated females are also presented.

Methods and materials. Adult hamsters weighing from 80 to 140 g were caged singly and given Purina chow pellets and water *ad libitum*. A bed of wood chips was placed in each cage. Throughout each study involving measurement of the same parameter, the following factors were constants: 1) age limits of animals used, 2) temperature of control room, 3) temperature of acclimation room, and 4) duration of acclimation period. In many of the studies some of these factors were altered, and these details are given with tabulated results in Tables I-V. Males were used exclusively, except in one study designed to compare cold-induced kidney growth in male and female hamsters.

At the end of the acclimation period, the animals were weighed, killed by decapitation, and bled. The kidneys were decapsulated, rinsed in cold tap water, rolled against damp filter paper, and weighed immediately. They were then minced and expressed through an ice-cold fine-grille stainless steel tissue press, and the resulting pulp homogenized in a Potter-Elvehjem iced tissue grinder. Tissues for DNA and total nitrogen assays were homogenized in distilled water. Isolation of microsomes was carried out in 8.5% sucrose

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TABLE I. Effect of Cold and Heat Acclimation on Body and Kidney Weights of Male Hamsters.

Group	No. animals	Age, wk	Ambient temp, °C	Body wt, g	Wet wt per pair of kidneys	Wt both kidneys/ Body wt × 100
Cold-acclimated, 4-6 wk	16	12-14	4 ± 1	96.4 ± 3.8*[1]	1.13 ±.025[4]	1.18 ±.028[7]
Controls	16	12-14	25 ± 2	120.1 ± 3.2[2]	.95 ±.018[5]	.78 ±.019[8]
Heat-acclimated, 4-5 wk	16	12-14	33 ± 4	114.1 ± 4.2[3]	.77 ±.020[6]	.68 ±.022[9]
Probability points				P 1,2 <.001	P 4,5 <.001	P 7,8 <.001
				P 2,3 <.1	P 5,6 <.001	P 8,9 <.01
Cold-acclimated, 8-10 wk	15	16-18	5 ± 1	111.8 ± 3.0	1.42 ±.03	1.29 ±.040
Controls	15	16-18	25 ± 1	146.6 ± 4.1	1.08 ±.025	.74 ±.012
Probability point				P <.001	P <.001	P <.001

* Mean ± standard error of mean.

TABLE II. Effect of Cold Acclimation on Total Nitrogen and DNA Content of Male Hamster Kidney.

Group	No. animals	Age, wk	Ambient temp °C	Wet wt in g per pair of kidneys	mg nitrogen per pair of kidneys	μg DNA per pair of kidneys
Cold-acclimated for 8 wk	5	13-15	5 ± 2	1.28 ± .06	36.78 ± .62	2457.0 ± 360
Controls	5	13-15	23 ± 2	.91 ± .04	28.37 ± 1.01	1793.2 ± 226
Probability point				P < .01	P < .01	P < .02

TABLE III. Effect of Cold Acclimation on Succinic Dehydrogenase, Succinoxidase and Transaminase Activity in Male Hamster Kidney.

Group	Age, wk	Ambient temp, °C	Succinic dehydrogenase*	Succinoxidase (μl O_2 per mg N/hr)	Transaminase (Frankel units)
Cold-acclimated for 3-5 wk	12-14	5 ± 2	41.6 ± 1.5 (17)	93.2 ± 6.2 (10)	60 ± 3.1 (7)
Controls	12-15	28 ± 4	28.3 ± 1.1 (17)	68.3 ± 4.4 (8)	59 ± 2.4 (7)
Probability point			P < .001	P < .05	P > .8

* μg of triphenyltetrazolium chloride reduced per mg (dry wt) per hr.

TABLE IV. Effect of Cold Acclimation on DPNH-Cytochrome c Reductase of Microsomes and on Total Microsomal Nitrogen in Male Hamster Kidney.

Group	No. animals	Age, wk	Ambient temp, °C	Activity of microsomal DPNH-cytochrome c reductase*	Total mg of microsomal nitrogen per pair of kidneys	Calculated total activity of DPNH-cytochrome c reductase per pair of kidneys
Cold acclimated for 8-10 wk	10	16-20	5 ± 2	1800 ± 90	1.19 ± .092	2140 ± 120
Control	9	16-20	23 ± 2	1910 ± 67	.84 ± .090	1608 ± 142
Probability point				P < .01	P < .02	P < .01

* △ O.D. at 550 mμ per mg N per hour.

TABLE V. Comparative Effect of Cold Acclimation on Kidney Growth in Male and Female Hamsters.

Group	No. animals	Age, wk	Ambient temp, C°	Body wt, g	Wet wt in g per pair of kidneys	% increase of kidney wt
Females cold-acclimated for 4 wk	6	11-14	5 ± 1	95.1 ± 5	1.23 ± .04	22.0
Female controls	6	11-14	23 ± 1	105.3 ± 2.3	1.01 ± .04	
Probability point				P < .01	P < .01	
Males cold-acclimated for 4 wk	4	11-14	5 ± 1	111.5 ± 4	1.11 ± .03	21.8
Male controls	5	11-14	23 ± 1	121.6 ± 6	.91 ± .04	
Probability point				P < .01	P < .01	

by standard centrifugation procedures in a Spinco Model L ultracentrifuge. For succinoxidase, succinic dehydrogenase, and glutamic-oxaloacetate transaminase assays, homogenizations were carried out in 0.1 M potassium phosphate buffer (pH 7.4).

Nitrogen assays were made using the micro-Kjeldahl method. DNA was assayed by the Dische method as modified by Schneider (4). Succinoxidase activity was determined manometrically employing the procedure described by Schneider and Potter(5). Succinic dehydrogenase activity was measured spectrophotometrically by the method of Kun and Abood(6) as modified by Hoch and Vallee(7). Transaminase was assayed using the Reitman-Frankel method(8). The above enzymatic assays were made at 37°C.

Detailed techniques for the assay of microsomal DPNH-cytochrome c reductase are described elsewhere(9), except that the reaction rates in this study were recorded on a Cary Model 14 M spectrophotometer at 26°C.

To study early mitotic activity, the kidneys of hamsters acclimated to 4°C for 48, 72, 96, 120 and 240 hours were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with Feulgen's reagent. For studies of kidneys from fully cold-acclimated hamsters (exposed for 2 months at 4°C) in which mitosis has all but ceased, the sections were stained with eosin and hematoxylin. Controls were maintained at the ambient temperature of 25°C.

Statistical evaluation of the data was made first by a comparison of variance, and then, where indicated, by group comparisons using the student t-test. Group differences where the probability point (P) is less than 0.05 were considered significant.

Result and discussion. After 4 to 6 weeks of cold-exposure, hamsters have a 24% lower body weight, but an 18% higher kidney weight than animals in the control room (Table I). The differences become greater if the cold-exposure period is extended 3 or 4 weeks, body weight becoming about 30% lower and kidney weight 33% higher. These changes are similar to, but less striking than those reported by Knigge(1), who found that hamsters exposed to approximately the same cold conditions had kidneys more than 60% heavier than those of controls. In any case, it is clear that cold-exposure induces a greater per cent weight increase in the hamster kidney than in the rat kidney(10,11). Whether this enhanced kidney growth is associated specifically with preparation for hibernation, or is merely a compensatory response to cold, remains to be determined.

In the albino rat, which exhibits stress symptoms when exposed to similar cold conditions(12), it is possible that the stimulation of mitosis in the renal tubules(13) results from increased adrenal cortical output, since some cortical steroids are known to stimulate kidney growth(14,15). However, in the case of the cold-exposed hamster, the renal growth-promoting role of the cortex is questionable, since this endocrine gland involutes during cold-exposure(16). Furthermore, hamsters stressed by heat sufficient to cause 20% mortality within a month have kidneys about 15% smaller than those of controls (Table I).

It is known that injections of testosterone stimulate kidney growth(17,18), but since female hamsters subjected to identical cold conditions have the same renal enlargement as the males (Table V), a change in the levels of circulating testosterone can be ruled out as being the stimulus for the observed renal growth.

Thyroid extracts can cause renal enlargement(19), as well as an increase in total DNA content of the kidney(20). However, it has not been conclusively demonstrated that thyroid activity is elevated in cold-acclimated hamsters. Knigge(21) has presented some rather convincing evidence that there is hyperthyroid activity in the cold-acclimated hamster, but conversely, other evidence seems to indicate that there may instead actually be a diminution(16,22,23). Thus the role of the thyroid in inducing the renal growth is not clear.

After the first 3 days of cold exposure, there are distinct histological changes in the kidney tubules. There are many more divid-

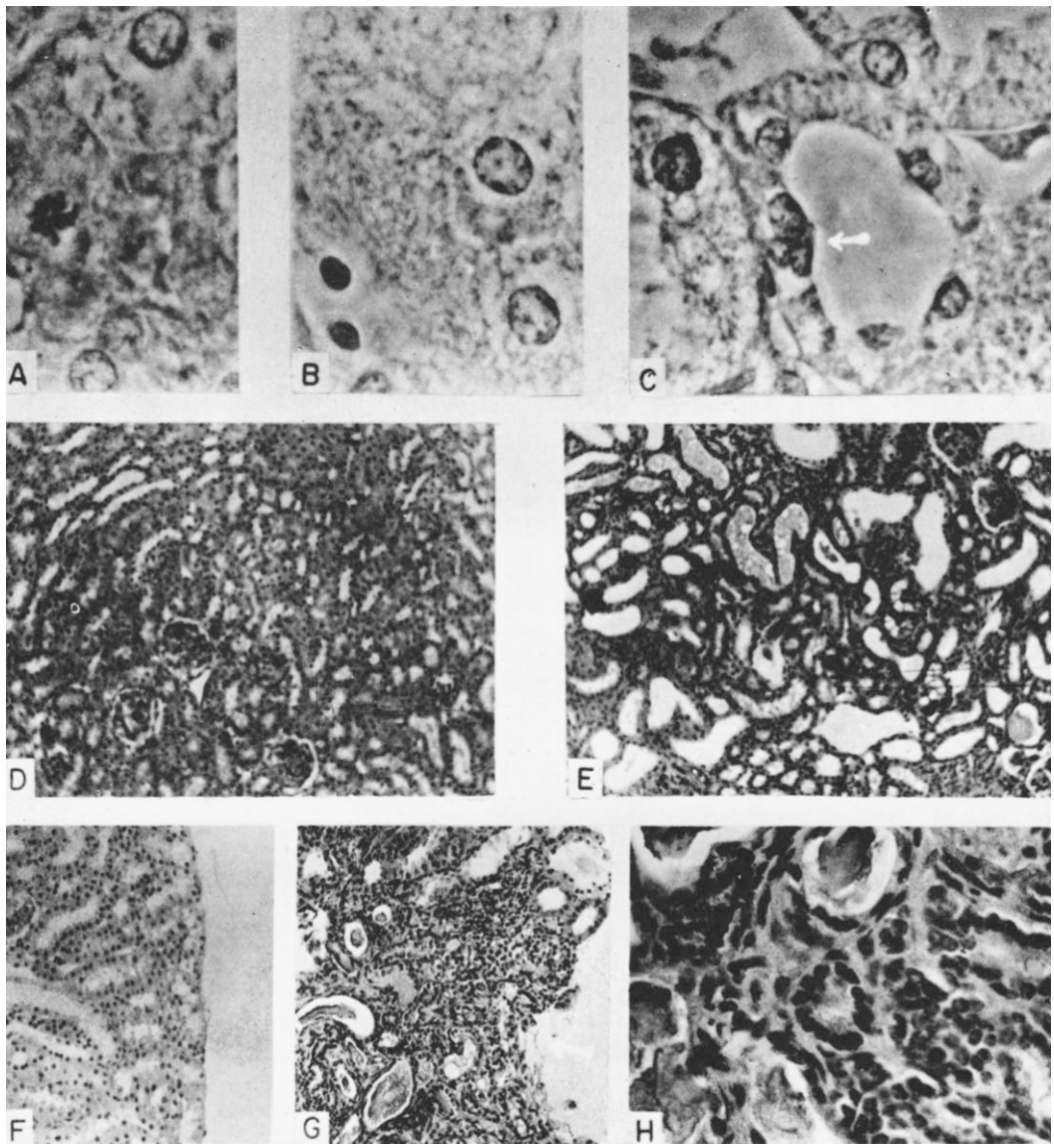

FIG. 1. Pictures of histological slides of kidney tissue from control and cold-exposed hamsters. A. and B. Mitotic figures seen in tubular epithelium after 48 hr of cold-exposure, 800×. C. Tetranuclear cell after 120 hr of cold-exposure, 800×. D. Cortical section of kidney of control, 80×. E. Cortical section of kidney of 60-day cold-acclimated hamster, 80×. F. Surface of kidney of control, 100×. G. Surface of kidney from 60-day cold-acclimated hamster showing a pit (arrow), 100×. H. Section of kidney of 60-day cold-acclimated hamster showing occluded tubule, 320×.

ing cells (Fig. 1, A and B) and multinucleate cells (Fig. 1, C) in the tubular epithelium of the cold-acclimated animals than in the controls, in which such cells are rare. Between the 96th and the 240th hour after the animals are placed in the cold, the number of mitosing cells remains about the same, but the number of binucleate and tetranucleate cells increases slightly. A few epithelial cells contain as many as 6 nuclei, and these cells extend into the lumina of the tubules as blebs. Mitosis in the multinucleate cells is interesting because nuclear division is stimulated, whereas division of the rest of the

cell either is not stimulated or is inhibited. What the factors may be which unbalance the normal sequence of events in mitosis presents an interesting problem.

After 8 to 10 weeks of cold-exposure, profound histological changes have occurred in the kidneys (Fig. 1, D *vs* E). There are more epithelial cells per sagittal section of kidney than is the case in the controls. A few of the tubular cells of the fully acclimated animal are multinucleate, whereas such cells are rare in the controls. On the other hand, mitosing cells are rare, as they are in the controls, indicating that cell division has diminished to the very low pre-acclimation rate.

The surface of the kidney of the acclimated animals is pitted, which is not the case in the controls (Fig. 1, G *vs* F). The tubules are very dilated in some areas and either diminished in size or completely occluded in others, whereas in the controls, tubule size is relatively uniform (Fig. 1, D *vs* E). The lumina of quite a few of the dilated tubules contain albuminous casts. The number of nuclei per glomerulus (Fig. 1, E *vs* D) and per cross-section of the tubules (especially in the proximal portion) of the cold-acclimated animal is noticeably greater than in the control. Fig. 1, H shows a cross-section of an occluded tubule from an acclimated animal in which the nuclei are so crowded that they appear to form a continuous ring with no visible cytoplasmic separation. The increase in nuclei per cross-section of tubule is also noticeable in dilated tubules. The tubules of the control are relatively uniform in size throughout the kidney. Alternating areas of hyperemia and ischemia, and increased hyaline degeneration and angiomatous stroma develop in the corticomedullary junction zone of the kidney of the acclimating animal. Although sclerosis *per se* is not detectable in most acclimated animals, it occurs in some. These characteristics are not detectable in the control kidney. The kidneys of cold-acclimated hamsters have a histological appearance strikingly similar to that characteristic of renal hypertension and mild ischemia. If renal hypertension in the hamster results from prolonged cold-exposure, both its initiating causes and its physiological effects on the whole organism are of interest.

One possible cause of the observed changes in the kidney of the acclimated animal might be a mechanical constriction and subsequent ischemia of the kidney, which possibly results from its rapid growth in a relatively confined space. In fact its histological appearance is similar to that of the kidney of a rabbit in which renal blood flow has been restricted(24). It is known that prolonged renal hypertension results in cardiac hypertrophy, and we have observed this condition in long-term cold-acclimated hamsters.

Tables II, III and IV summarize the results of biochemical assays on the kidneys. Total DNA and total nitrogen per kidney are 44% and 30% greater, respectively, in cold-acclimated than in control animals (Table II). However, since much of the nitrogen in the kidney of the acclimated animal is in the albuminous casts, the actual increase in cellular nitrogen may be considerably less than 30%. These biochemical data correlate well with the histological picture in that both indicate a greater per cent increase in nuclear material than of total cellular protein. However, the 42% increase in "microsomal" nitrogen (Table IV) might be interpreted as an index of the increase in endoplasmic reticular membranes and associated ribosomes, which would be compatible with the overall increase in protein synthesis necessary for the observed growth.

Although the *specific* activity of kidney microsomal DPNH-cytochrome c reductase activity is lower in the acclimated animal than in the control, *total* activity per kidney is elevated (Table IV). The low specific activity might be a consequence of the fact that the activity is expressed per milligram of microsomal nitrogen. Since there are many other nitrogen-containing compounds associated with other microsomal functions, for example those enzymes and RNA associated with protein synthesis, these contribute to the milligrams of nitrogen upon which the specific DPNH-cytochrome c reductase ac-

tivity is based, and thus could account for its relatively low specific activity values in the acclimated animal.

It has been hypothesized that these pyridine nucleotide-cytochrome c reductases may be calorigenic *in vivo*(25) as they have been shown to be *in vitro*(26). We have found an elevation of DPNH-cytochrome c reductase in the liver microsomes of the cold-acclimated hamster(27), and this has been found also by Smith(28) and by Beyer(29) in the case of liver microsomes from cold-acclimated rats. Thus, the increase in total kidney DPNH-cytochrome c reductase in the kidney indicates that an elevation of this calorigenic system is not confined to liver tissue, and it may be of significance in contributing to the overall heat production of the hamster in the cold.

Kidney succinic dehydrogenase and succinoxidase activity is higher in the acclimated animal than in the control (Table II). These increases in activity, however, are not nearly so striking in the kidney as in the liver(22). Nevertheless, the fact that these kidney oxidative enzymatic systems are significantly elevated indicates an increased potential for oxidation of Krebs cycle intermediates.

It is clear from the observed kidney growth that protein synthesis is stepped up in the kidney during acclimation, and it might be supposed that transaminase activity would be greatly enhanced, as Klain(30) has found in liver of the cold-acclimated rat. However, no measurable increase in transaminase activity apparently occurs in the hamster kidney (Table III). Since a high concentration of blood-borne amino acids is available to the kidney during acclimation, its rapid growth may involve primarily an assemblage of these, with little extra amino acid synthesis or degradation.

Summary. The effects of cold acclimation on the kidney of the adult male hamster *(Mesocricetus auratus)* were studied. The animals were exposed to an ambient temperature of 4°C for periods of 4 to 10 weeks. Biochemical changes induced by cold acclimation include: 1) increased succinoxidase and succinic dehydrogenase activity, 2) increased total microsomal DPNH-cytochrome c reductase activity per kidney, but a decreased specific activity of this enzyme, and 3) increased total nitrogen, microsomal nitrogen, and DNA. The activity of glutamic-oxaloacetate transaminase is unchanged. The wet weight of the kidney is much greater in cold-acclimated than in control animals, but decreases in heat stress. Thus, renal growth in the cold-acclimated animals probably is not simply a response to stress. Kidney growth is the same in cold-acclimated males and females, indicating that gonadal hormones are not specifically involved. Striking histological changes occur very soon after the animals are exposed to cold. After 2 days of cold-exposure, mitotic figures and multinucleate cells appear, especially in the proximal tubule. Between the fourth and tenth days, the number of mitosing cells remains fairly constant, but the number of multinucleate cells increases. Quite a few binucleate and, less frequently, tetranucleate cells appear. In a few cells, clusters of 6 nuclei were observed. After 8 weeks of exposure, mitosis has ceased and multinucleate cells are very uncommon. The tubules are dilated in some areas, diminished in size or, quite frequently, even occluded in others. These areas alternate, and probably are areas of hyperemia and ischemia, respectively. There is hyaline degeneration and angiomatous stroma in the cortico-medullary junction zone. Albuminous casts are common. The number of cells in the glomeruli is conspicuously high. The kidneys appear to have quite a few of the classic characteristics of renal hypertension, but sclerosis *per se* is not detectable in most of the acclimated animals.

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Stimulation of *Turbatrix aceti* by Antibiotics. (28295)

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The mechanism of action of antibiotics in increasing growth rates, food efficiency, and other parameters of biological systems has been debated with varying degrees of evidence(1) since the discovery of growth stimulation(2). One of the main difficulties is the inadequacy of methods. Problems with vertebrate experimentation involve space, time, complexity of the organism, and variability. The main difficulty with the bacterial tool is the separation of mutation from adaptation. Consequently an invertebrate species was sought in an effort to combine the speed and power of bacteriological methods with the significance and ease of separation of adaptation from mutation presented by metazoan species. Of several invertebrates considered, the vinegar eel, *Turbatrix aceti,* has many assets: its small size, its availability in large numbers, ease of handling as a suspension, and ease of obtaining sterile material. The data presented here indicate that different antibiotics stimulate this species over a wide range of concentration.

Methods. Turbatrix aceti from unprocessed vinegar obtained from a vinegar factory, Établissements Lourtie at Theux, Belgium, were maintained in vinegar adjusted to pH 5.0 at room temperature. Procaine penicillin, oxytetracycline Cl, chloramphenicol, and staphylomycin were dissolved in vinegar at pH 5.0. The staphylomycin was first dissolved in a small quantity of methanol. In each experiment antibiotics were added in graded levels to flasks containing vinegar at

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