

Ageing and the Regulation of Cell Activities during Exposure to Cold

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ABSTRACT The inability to maintain body temperature and a selective pattern of changes in the regulation of cell activities were revealed by briefly exposing ageing C57B1/6J male mice to cold (10°C). The induction of liver tyrosine aminotransferase (TAT) during exposure to cold (a gene-dependent process) was markedly delayed in senescent mice (26 months old) as compared with younger mice (3–16 months old); after the delay, the rate of increase of TAT was similar to that prevailing in younger mice. Direct challenge of the liver with injections of corticosterone or insulin elicited the induction of TAT on an identical time course in young and senescent mice. These experiments provide an example of an age change in a gene-dependent cell process (the delayed induction of TAT in senescent mice during exposure to cold) which is not due to a change in the potential of the genome for responding when exogenous stimulæ are supplied (injection of hormones). In contrast to the age-related change in liver cell activities, no significant changes were found in the secretion of corticosterone during exposure to cold. Although the seat of these selective age-related changes in the regulation of cell activities remains unclear, it is argued that generalized damage to the genome of cells throughout the body is not involved. The results of this and other studies showing the selective effect of age on cell activities are considered in terms of the concept that many cellular age changes represent the response of cells to primary age-related changes in humoral factors in the internal environment of the body.

INTRODUCTION

The effect of age on thermoregulation and on activities in the liver and adrenal cortex of mice during exposure to cold is described in this report; we find that age has a selective effect on cell activities in these organs. The essence of ageing is commonly considered to be a process of random damage to cells, proceeding independently in cells throughout the body. However, selective age changes in integrated physiological processes (e.g., thermoregulation) are not readily interpreted by this concept. In order to adequately describe our experiments we will first review certain studies which support

an alternative concept, namely that humoral factors play an important role in mediating changes in cell activities throughout the body during ageing.

Characteristic of many plant and animal species is a maximum longevity; even in the most carefully managed populations (e.g. a germ-free mouse colony (Gordon et al., 1966)), the rate of mortality increases exponentially after a certain age, thereby determining a maximum longevity characteristic of the species (Comfort, 1964). In contrast, a number of species do not appear to have a characteristic maximum life-span, e.g. the redwood (*Sequoia sempervirens*), certain hydroids (*Cyanea capillata*) (Brock and Strehler, 1963), and possibly some tortoises (*Testudo sumeiri*) (Comfort, 1964); individuals of such species may be considered potentially immortal. Because of a lack of detailed information, it is not known how widely such species are distributed in nature.

The increasing rate of mortality in those species with a characteristic maximum longevity (e.g. man and other mammals) has been attributed to a progressive impairment of cell activities, the result of a presumed accumulation of random mutations independently in cells throughout the body (Szilard, 1959; Wulff et al., 1962; Crowley and Curtis, 1963). However, certain mammalian cell activities remain unimpaired throughout adult life. For instance, the output of pituitary gonadotrophins can be maintained at up to 10-fold normal levels in human eunuchs over a period of nearly 50 yr (Hamilton et al., 1945). The rate at which the liver can regenerate its mass following partial hepatectomy is also maintained throughout life in the rat (Bucher and Glinos, 1950; Bourlière and Molimard, 1957). Moreover, the potential of diploid lung fibroblasts for proliferation in vitro is indicated from Hayflick's studies of cell culture in vitro to be similar in tissue from adult human donors ranging in age from 22 to 87 yr (Hayflick, 1965; Hayflick, 1968). These varied examples imply that a *generalized* impairment of cell activities does not occur during ageing. In fact, it appears that age-related changes are *highly selective*, e.g. the turnover rate of intestinal epithelial cells in the mouse is retarded in the duodenum (Leshner et al., 1961), but not in the ileum (Fry et al., 1962); and the growth rate of hair in human males is retarded on the chin, but not on the eyebrows (Myers and Hamilton, 1961). Thus, activities in each cell type appear to change during ageing in a specific and characteristic way.

Age changes in at least some mammalian cell activities can be reversed. For instance, the slowed turnover of intestinal epithelial cells in senescent rats can be accelerated by radiation exposure to the same rate prevailing in young rats during radiation exposure (Leshner, 1966). In addition, the slowed regrowth of hair in senescent mice may be restored to the rate of hair regrowth in young mice by transplantation of skin from a senescent to a young mouse (Horton, 1967); this result implies that changes in the regulation of humoral

factors are important in mediating age-related changes in mammals. The reversibility of age changes in these cell types does not support the notion that ageing results from damage to the genomic apparatus. The occurrence of selective and reversible changes in cell activities during ageing indicates that *random* damage to the genomic material in cells throughout the body is not likely to be the primary cause of ageing. It is nonetheless possible that *certain* key cell types are susceptible to random genomic damage; disturbances in these cells might in turn affect cells throughout the body via humoral factors, e.g., as is proposed in the immunological theory of Burch (1968).

A definite change in humoral factors of the internal environment during ageing is the cause of age-related changes in cell activities in some vertebrate species. The histological and pathological changes which precede death at the first spawning of five species of Pacific salmon (genus *Oncorhynchus*) are a well-documented result of adrenal corticosteroid toxicosis (Hane and Robertson, 1959; Robertson and Wexler, 1960; Robertson et al., 1961 *a,b*); similar changes can be produced by implantation of hydrocortisone pellets in the rainbow trout (*Salmo gairdnerii*), a species which normally survives the first spawning (Robertson et al., 1963). Furthermore, the elaboration of toxic quantities of adrenal corticosteroids can be prevented in Pacific salmon by castration of immature fish; the longevity of the survivors of this operation may be nearly 100% greater than the normal life-span limit of this species (Robertson, 1961). A similar example is provided by the European eel (*Anguilla anguilla*) which matures in the freshwater streams of Western Europe. At a certain point, eels begin a remarkable, 3000 mile passage to the Sargasso Sea where spawning occurs. No food is eaten during this journey, the gastrointestinal tract atrophies, and general demineralization of bony structures occurs (Bertin, 1956). Upon spawning, the eels die. However, if maturing eels are prevented from returning to the sea, this involution does not occur and their life-span can be greatly extended above its natural limit of about 15 yr: there are at least four authenticated cases of European eels which achieved a life-span of 50 yr or more (Bertin, 1956; Lekholm, 1939; Vladkov, 1956). In both these teleost species, altered cell function during ageing is associated with changes in humoral factors of the internal environment [hyperadrenocorticism and its sequelae in the Pacific salmon (Robertson et al., 1961 *a*); inanition and shifts in blood electrolyte concentrations in the European eel (reviewed in Bertin, 1956)]. Thus, many age changes in these teleosts result from the response of cells throughout their bodies to primary age changes in humoral factors. The reversibility of age changes in the rate of hair regrowth upon the transplantation of skin from a senescent to a young mouse (Horton, 1967) implies that humoral factors are also important in mediating age-related changes in mammals. The particular age-related changes in humoral factors which occur in the Pacific salmon and the Euro-

pean eel are obviously not typical of ageing in mammals, e.g., basal levels of electrolytes and of most hormones in the blood, etc. do not appear to change substantially with age in mammals.

The escape from the normal maximum life-span which is possible in Pacific salmon and European eels argues powerfully against the importance of damage inflicted on the cell by time-dependent events, e.g., the gradual accumulation of mutations. A similar conclusion may be drawn from experiments of Krohn, in which mouse skin was serially transplanted through a sequence of young host mice until the transplanted skin reached the age of 80 months (about twice the maximum longevity of mice) without manifesting degenerative changes (Krohn, 1966).

In conclusion, the maximum life-spans of the Pacific salmon, the European eel, and of various mammalian species do not seem to be determined by the accumulation of genomic damage in cells throughout the body. In the Pacific salmon, changes of regulation in certain neuroendocrine axes (gonad-hypothalamus-pituitary-adrenal cortex) affect humoral components and clearly cause "natural death" in these species.

In the experiments described below, we present an analysis of thermoregulation in the ageing mouse. Body temperature is a crucial parameter of the internal environment in homeothermic mammals and is well-known to be under neuroendocrine control. The agency of exposure to cold was found to provide a suitable stress for revealing age-related changes in thermoregulation and in the regulation of certain cell types known to be involved in mammalian thermogenesis.

MATERIALS AND METHODS

1. *Description of the Mouse Colony*

The C57B1/6J male mouse was selected for this study because of its lifelong vigor and the relatively low incidence of neoplasms and other pathological conditions (Storer, 1966; Russell, 1966). A detailed description of our colony of ageing mice is given below.

SOURCE AND HUSBANDRY OF MICE All mice were obtained from Jackson Laboratory, Bar Harbor, Me. Retired breeders (8 months old) were received in shipments of 20 and were caged at random in groups of 5. The mice were maintained in a room exclusively for the ageing colony and for other young C57B1/6J male mice, 3-4 months old, which were held at least 1 month after acquisition before use; entrance was restricted to the experimenters and to maintenance personnel. The room was kept at a constant temperature (74-78°F); fluorescent lights were scheduled to go on at 7 a.m. and off at 7 p.m. Food (Purina Lab Chow, which contains 23.4% protein and 4.3% fat) and tap water were provided ad lib. except as indicated. Cages with Lab Litter (Carworth, Inc., New City, Rockland County, New York) were changed and sterilized once weekly; water bottles were changed and sterilized twice weekly.

IDENTIFICATION OF THE ONSET OF SENESCENCE The onset of senescence (the terminal phase of life) may be characterized by the conspicuous increase of disease and mortality which is known to occur in populations of ageing mammals sometime after maturity (Jones, 1956; Simms and Berg, 1957). In the present study, maturity is defined as the age at which maximum body weight is reached (about 5–7 months in our colony); as described below, body weight is generally stable thereafter. As the pattern of mortality and incidence of disease at any chronological age can vary markedly from population to population (Jones, 1956; Russell, 1966), the rate of mortality characteristic of another population of C57B1/6J male mice could not be considered a priori to apply to our colony. Hence, a great deal of attention was given to the state of health of the mice and the incidence of mortality in our colony, so that the age of onset of senescence could be recognized.

TABLE I
WEIGHT CHANGES BEFORE DEATH

Class of change	No. of mice	Total
		%
Mice without weight change	23	60
Mice with weight gain (usually bearing tumors of mesenteric lymph nodes* and frequently attended by hypertrophy of the liver; in such cases the liver was often grossly enlarged (weighing up to 3.5 g†), pale, and fibrotic)	6	15
Mice losing 10% or more body weight over a period longer than 6 wk before death	10	25

* This is a characteristic malignancy of C57B1 derived strains of mice (Murphy, 1966; Russell, 1966).

† The normal liver weight of mice in our colony (previously fasted for 20 hr) at the age of 8 months and 26 months is 1.2 ± 0.05 g or $4.3 \pm 0.12\%$ (mean \pm SEM, 12 mice) of body weight.

The health of the mice in the colony was excellent during most of their life-span, as judged by the following criteria: stability of body weight; the absence of ecto- or endoparasites, *Salmonella*, or of pathological conditions; and normal values of hematocrits, of differential blood cell counts, of serum osmolality, and normal distributions of plasma proteins upon cellulose strip electrophoresis (Finch and Foster, manuscript in preparation). A description of liver histology in different age groups is given in Appendix I.

Although large and progressive losses of body weight before death (extending over a 3–4 month period) have been observed in ageing white rats (Everitt, 1957), monthly weighing of each mouse in our colony did not reveal any general loss of body weight before death up to the age of 30 months. (The average body weight of randomly sampled mice was 28.9 ± 0.3 g at 10 months and 29.4 ± 0.6 g at 26 months (mean \pm SEM, 11 mice).) Table I shows the pattern of body weight change before death in the group of mice whose pattern of mortality is described in Figs. 1 and 2.

The age specific mortality rate remained low until after reaching the age of 21–23

months. On the average, 50% of any cohort (mice with the same birth date) survived until the 28th month (Fig. 1). Cohorts of mice 25 months or older are clearly in a phase of rapidly increasing mortality (Fig. 2). 10% of the mice in the cohort depicted in Figs. 1 and 2 eventually survived to the age of 36 months and 2% survived to the age of 40 months (not shown in Figs. 1 or 2). The mortality characteristics of the mice

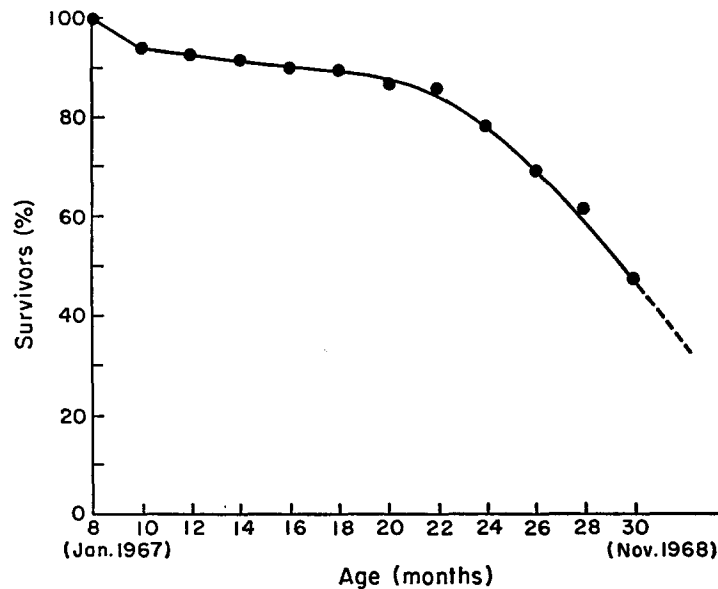


FIGURE 1 (top). Survival of the control group as a function of age. The data were determined from colony records of a group of 96 mice.

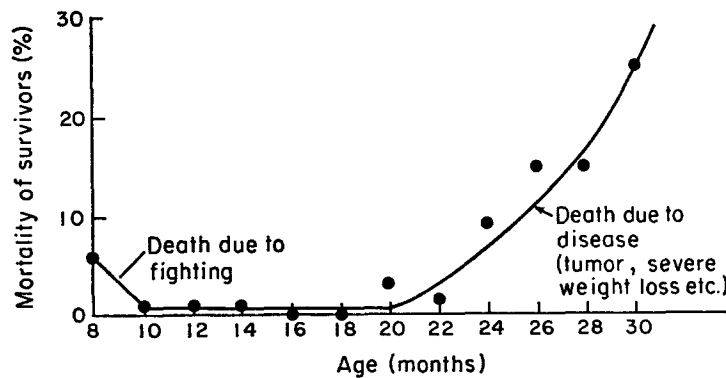


FIGURE 2 (bottom). The death rate of the mice in Fig. 1 as a function of age. This graph represents the per cent of survivors at any age which died during the preceding 2 month interval (calculated from Fig. 1). The deaths due to fighting occurred in the first week the mice were received from the supplier; the aggressive mice were identified and separated. A more complete description of the deaths due to disease is contained in the section on Materials and Methods.

in our colony compare favorably with other colonies of C57B1/6J male mice (Russell, 1966; Goodrick, 1967).

The incidence of pathological changes (tumors, severe weight loss, etc.) in any cohort increased concurrently with the increase of mortality in that cohort (e.g. at necropsy of mice sacrificed during experiments, obvious pathological changes were found only in 2% of mice aged 15–18 months, whereas about 15% of mice aged 28 months were abnormal). The close relation between the increase of disease and mortality during ageing has been well-documented in rats by Simms and Berg (1957). The mortality rate in all cohorts was a strict function of chronological age: as shown in Fig. 3, the mortality rate of a slightly younger group of mice (caged nearby) increased *only* after reaching a certain age (21–23 months). Thus, the concurrent increase of varied pathologic changes with the increase in mortality cannot be the result of an ordinary episode of communicable disease (e.g. an epizootic of the much feared *Salmonella typhimurium*), but they are definitive characteristics of senescence in a mammalian population (Simms and Berg, 1957; Jones, 1956). Cohorts of mice aged 25 months or older (which always manifested a rapidly increasing rate of mortality) were therefore designated as senescent.

In any age group, cages of mice were randomly selected for experiments.

2. Assay of Tyrosine Aminotransferase

Tyrosine aminotransferase (1-tyrosine:2-oxoglutarate aminotransferase, E.C.2.6.1.5), or TAT, was assayed according to Lin and Knox (1957); tautomerase (beef kidney, grade I) was obtained from Sigma Chemical Co., St. Louis, Mo., and all batches were found to have negligible TAT activity. The final product of the enzyme reactions, the enol-borate complex of *p*-hydroxyphenylpyruvic acid, was identified by its characteristic E_{\max} at 310 m μ . The assay was conducted in a water-jacketed cuvette holder in covered 1 cm² cuvettes at 34°C.

Tissue was taken from the distal portion (0.2 g) of the right median lobe of the liver and immediately frozen on dry ice. The frozen tissue was ground in a room at 2°C with six double strokes of a motor-driven TenBroeck homogenizer to a cell-free homogenate in 8.0 ml of cold 0.14 M KCl. The supernate of 5,000 g/10 min centrifugation at 2°C contained 95% of the total TAT activity in the homogenate. No age-related differences in extractability of the enzyme from liver tissue were found. Activities of extracts in different proportions from young and old livers were additive.

TAT activities were expressed per unit DNA, a more reliable standard than liver protein, according to Grossman and Mavrides (1967). The above centrifugal pellet (consisting principally of intact nuclei) was washed once with cold 0.25 N PCA. Half-normal PCA extracts (70°C/30 min) of the washed pellet were found by Burton's modification of the diphenylamine reaction (Burton, 1962) to contain 98% of the DNA in the homogenate; the characteristic spectrum of the diphenylamine reaction with pure DNA (E_{\max} 600 m μ ; OD 600 m μ /640 m μ = 2.1) was obtained in these extracts.

All assays were performed in duplicate and gave highly reproducible values ($\pm 5\%$). The variations in TAT levels therefore appear to represent true individual differences.

3. Conditions of Exposure to Cold

Mice were placed singly (Grad and Kral, 1957) in prechilled, 2-qt glass jars. No food, water, or nesting material was provided during the exposure to cold.

In order to minimize individual differences in the pattern of feeding, a 20 hr fast preceded the experiments to be described. A 20 hr fast will eliminate stores of liver glycogen which could vary according to the recentness of feeding (Ekman and Holmgren, 1949). Large glycogen stores in the liver would be expected to alter the response to cold.

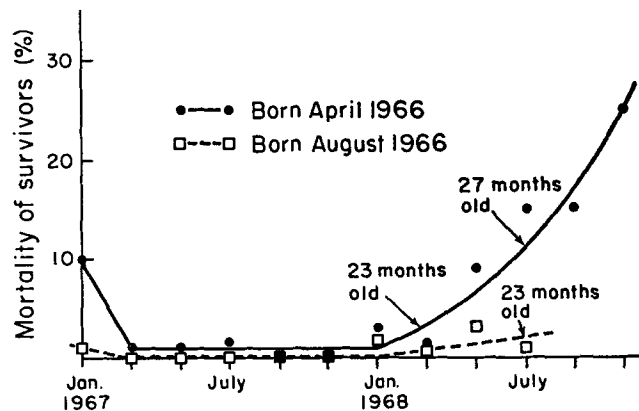


FIGURE 3. Death rate as a function of age in two groups of mice. The data have been calculated as described in the legend of Fig. 2 for two groups of mice. The mice born April, 1966, were previously described in Figs. 1 and 2. The mice born August, 1966, numbered 99 in January, 1967. It is evident from this graph that the death rate remains low for many months and increases noticeably only after a certain age (21–23 months).

All experiments were performed between 9 a.m. and 1 p.m., a period when diurnally varying TAT levels are known to be subject to only slight increases in young, fasted mice (Finch et al., 1969) which are of a lower order of magnitude than the changes observed in mice of all ages in the experiments described in the present paper. Mice of all ages are observed to be asleep or quiescent during the hours 9 a.m. to 1 p.m.

4. Colon Temperature

A lubricated thermocouple was inserted 4 cm through the anus into the colon after first causing defecation by abdominal massage as cautioned by Barnett (1956).

5. Corticosterone Assay

Corticosterone was assayed by the method of Peterson (1957) in duplicate aliquots of serum from blood collected from the jugular veins immediately after the mice were killed by cervical dislocation.

6. Reagents

Insulin (Iletin, Ely Lilly and Co.) was prepared in 0.14 M NaCl. Corticosterone (Mann Fine Chemicals, Inc., New York) was prepared from a concentrated solution in ethanol which was diluted into warm 0.14 M NaCl just before use. Solutions of both hormones were injected intraperitoneally with a 23G1 needle (0.75 IU insulin/kg body weight; 15 μ g corticosterone/kg body weight).

RESULTS

A. Body Temperature Regulation during Exposure to Cold

Ageing C57B1 mice are known to have difficulty in becoming acclimated to cold (Grad and Kral, 1957). We have found in particular that there is an impairment of thermoregulation during ageing in C57B1/6J mice from our colony. The colon temperature of young and senescent mice (previously fasted for 20 hr) was measured during brief exposure to cold as shown in Figs. 4 and 5. The average colon temperature of some fasted young mice actually increased during the first hour at 10°C, and declined only slightly thereafter, if at all. In contrast, there was a steady and progressive loss of body temperature in all senescent mice. It may be significant that the senescent mice with the lowest initial body temperatures suffered the greatest loss of temperature during exposure to cold. The initial average body temperature of both age groups was subnormal because of the fast which preceded these experiments (Finch, 1969). Marked changes in the ability to maintain body temperature have also been observed in ageing rats subjected to hypobaria (Flückiger and Verzar, 1955).

The increased thermogenesis elicited by exposure to cold (at least in young mice) results from a change in the pattern of metabolism in many organs of the body. We next examined cell activities in the liver and adrenal cortex, cell types long recognized for their role in regulating metabolic processes in the body, e.g. gluconeogenesis, which contribute to thermogenesis.

B. Cell Activities in the Liver (Studies of the Regulation of TAT)

Age-related changes in the regulation of liver cell activities were revealed in a study of the liver enzyme tyrosine aminotransferase (TAT). TAT is known to be inducible by the direct action of a variety of hormones (glucocorticoids, insulin, and glucagon (Goldstein et al., 1962; Hager and Kenney, 1968)) on the liver and is considered to have an important role in regulating gluconeogenesis (e.g. Feigelson and Feigelson, 1966), a metabolic process relevant to thermogenesis. The induction of TAT depends on gene activity (Greengard and Acs, 1962) and the selective synthesis of new TAT molecules (Kenney, 1962; Hager and Kenney, 1968). We have demonstrated for the first time that a rapid, gene-dependent induction of TAT occurs during stressful expo-

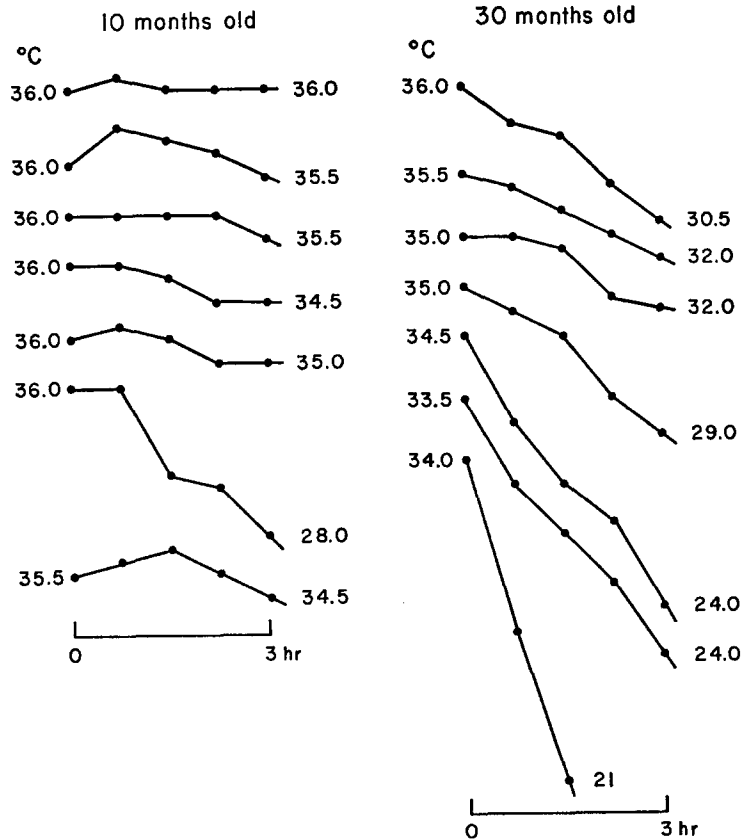


FIGURE 4. The effect of age on colon temperature during cold stress (individual records). Mice were fasted 20 hr and were exposed to cold as described in Materials and Methods. Colon temperatures were measured by inserting a thermocouple 4–5 cm through the anus of mice before and during cold stress at 9–10°C.

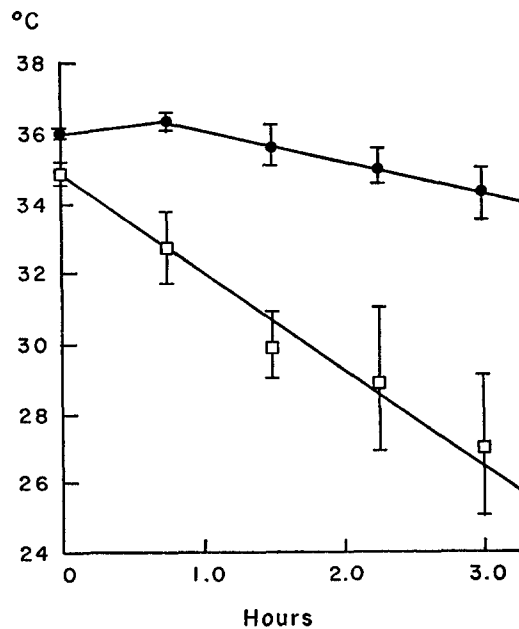


FIGURE 5. The effect of age on colon temperature during cold stress (9–10°C) (average values). The data are calculated from Fig. 4. Solid circles indicate 10-month old mice; open squares, 30-month old mice; I \pm SEM, 7 mice per age group.

sure to cold in young, fasted mice (Finch et al., 1969). The sensitivity of the cold stress-mediated induction of TAT to endogenous factors in the mouse (Finch et al., 1969), renders it an excellent probe for detecting changes in the regulation of humoral and other factors in internal environment.

1. DEPENDENCY OF TAT INDUCTION DURING EXPOSURE TO COLD ON GENE ACTIVITY IN SENESCENT MICE

The dependency of TAT induction during exposure to cold on gene activity in senescent mice was established in an experiment shown in Table II. Fasted senescent mice (29 months old) were injected intraperitoneally with actinomycin D¹ (2 mg/kg body weight in a solution of 0.14 M NaCl) 15 min before exposure to 10°C. Preliminary studies demonstrated that this dose of actinomycin D is more than sufficient to reduce the incorporation of ³H-orotic acid into high molecular weight, phenol-purified liver RNA by 95% in 8 month old mice within 15 min, in agreement with other published studies (Trakattellis et al., 1964). Thus, the induction of TAT in fasted senescent mice during exposure to cold requires DNA-dependent RNA synthesis (hence gene activity) as it does in fasted young mice (Finch et al., 1969).

2. DELAYED INDUCTION OF TAT DURING EXPOSURE TO COLD IN SENESCENT MICE

The effect of age on TAT induction during exposure to cold was demonstrated in a series of experiments with fasted mice of various ages. An illustrative experiment is shown in Fig. 6. In young adults and middle-aged mice² (at least until the age of 16 months), an increase of TAT activity was detectable at the earliest times sampled (30 min) and proceeded as a linear function of time from the beginning of the exposure to cold. However, in senescent mice (26–28 months old) there was a pronounced lag (in this experiment, about 120 min) before an increase of TAT activity could be detected; after this lag, the increase of TAT was a linear function of time. In five such experiments, the duration of the lag varied somewhat; in any experiment, the mice of a particular age group responded uniformly. The data from these five experiments may be summarized by the statements: (a) senescent mice (26 months or older) required 70 ± 20^3 min longer than younger mice (3–16 months old) to increase liver TAT during exposure to cold to a statistically significant level ($P < 0.05$) with respect to the initial values of that age

¹ Actinomycin D was generously provided by Mr. W. B. Gall of Merck, Sharp & Dohme, Rahway, N. J.

² The experiment represented in Fig. 6 included a group of middle-aged mice (16 months old) which was omitted from Fig. 6 for purposes of clarity (see Finch, 1969).

³ Mean \pm SEM.

TABLE II
THE INHIBITION BY ACTINOMYCIN D OF THE
INDUCTION OF TAT DURING EXPOSURE TO COLD IN
SENESCENT MICE

Group	TAT/DNA*	Range	No. of mice
Controls	$3.5 \pm 0.6 \times 10^{-2}$	2.0-4.5	4
Cold-treated	$10.0 \pm 0.3 \times 10^{-2}$	9.0-10.5	4
Actinomycin-treated, then cold-treated	$2.3 \pm 0.5 \times 10^{-2}$	1.0-3.0	5

All mice were sacrificed 4 hr after the start of the experiment.

* Mean \pm SEM.

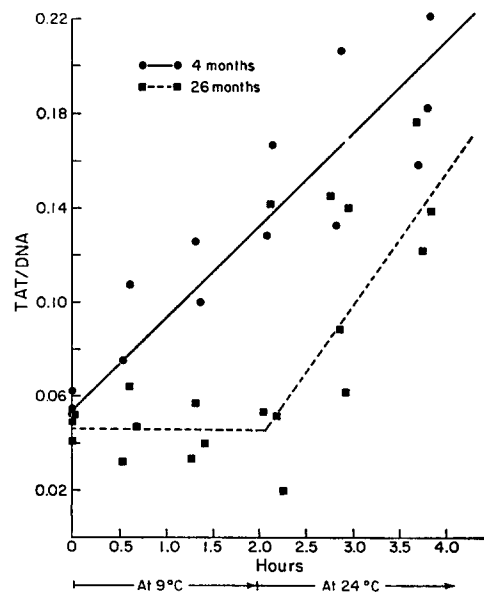


FIGURE 6. The effect of ageing on the induction of TAT by cold. Mice fasted 20 hr. Exposure to cold (9°C) begun at 9 a.m. After 2.0 hr all jars containing mice were removed to a room at 24°C to avoid widespread collapse of the senescent mice. The coincidence of the end of the lag in TAT induction in senescent mice with their removal from the cold did not occur in any other experiments. The enzyme activity is expressed per unit DNA (see Materials and Methods). The graphed function describing the changes of TAT was determined by computer analysis as the best fitting, broken-line function of the form ($y = a, t < \alpha; y = a + bx, t \geq \alpha; \alpha \geq 0$, in which $y = \text{TAT/DNA}$, $x = \text{time}$, b is the coefficient of TAT increase, $\alpha = \text{the lag}$). We are indebted to Mr. Bruce W. Knight, Jr., Affiliate of The Rockefeller University, for providing the computer program. Details of the program and of the calculations are given in Finch (1969).

group,⁴ (b) after this initial delay, the increase of TAT in senescent mice occurred at a rate closely similar to that in younger mice.⁵

3. THE ABILITY OF LIVER CELLS IN SENESCENCE FOR RAPID GENE-MEDIATED RESPONSES

The above results lead one to question the ability of the liver cell genome in senescent mice to respond rapidly to the endogenous stimulæ which operate during exposure to cold in fasted, younger mice. The ability of liver cells to respond rapidly to at least some hormones was verified by challenging the liver in fasted mice with intraperitoneal injections of insulin and corticosterone, hormones chosen for their ability to induce TAT in the isolated, perfused rat liver (e.g. Goldstein et al., 1962; Hager and Kenney, 1968). As shown in Fig. 7, a rapid induction of TAT resulted from injections of either insulin or corticosterone into young and senescent mice during the same span of time when lags were observed in senescent mice during exposure to cold. In two experiments with corticosterone and in two experiments with insulin, no age difference was found in the time required to achieve a statistically significant increase ($P < 0.05$) of TAT with respect to the initial values of the particular age group after induction by injected hormones.

The above result provides a very sensitive test of liver cell competence and is dependent on the normal function of particular gene control mechanisms and on the synthesis of both RNA and protein. If general damage to the cell nucleus were the primary cause of senescence, it might have been manifested during such a delicate process. In any case, the delayed induction of TAT during exposure to cold in senescent mice does not result from a loss of the ability of the liver to rapidly complete the complex process of enzyme induction but instead appears to reflect extrahepatic age changes.

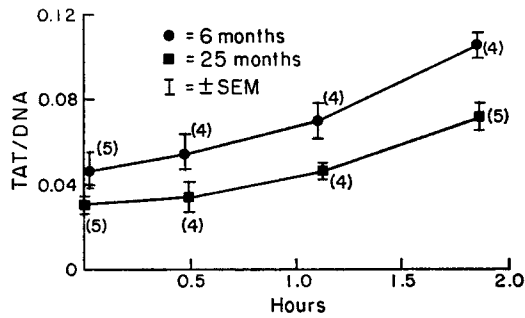
4. THE EFFECT OF AGE ON FASTING TAT LEVELS

Another effect of age on the regulation of TAT is revealed in Fig. 7. TAT levels initially and throughout induction by injection of insulin or corticosterone are significantly greater ($P < 0.05$) in young mice (4–9 months old) than in senescent mice (26 months old). Examination of mice of intermediate ages (Table III) revealed that fasting TAT levels are highest in young mice,

⁴ Essential for these calculations are the facts that (a) the coefficient of variation, σ/\bar{x} , was not different in the control values for TAT in any age groups; (b) σ/\bar{x} did not increase in mice sampled during the course of the experiments except momentarily at the termination of the lag during exposure to cold in senescent mice (Finch, 1969).

⁵ The rate of increase of TAT during the ascending phase of induction is 0.045 ± 0.003 TAT/DNA per hr in young mice and 0.045 ± 0.010 TAT/DNA per hr in senescent mice. These rates are the mean values \pm SEM of the best fitting slopes calculated from the data of five experiments, as described in the legend of Fig. 6.

but are 30–40% less in middle aged and older mice ($P < 0.01$). Thus, the decrease of fasting TAT levels occurs at a younger age than that at which the delayed induction of TAT is manifested during exposure to cold.



Induction of TAT by Insulin

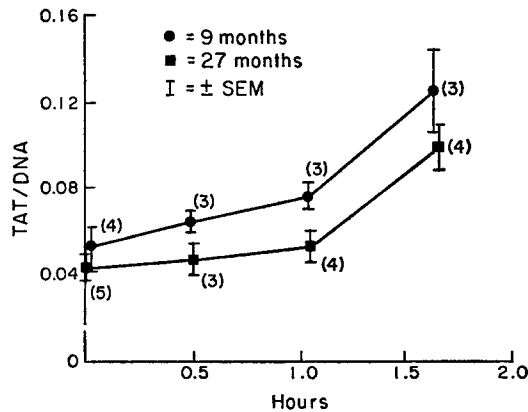


FIGURE 7. The effect of ageing on the induction of TAT by insulin and corticosterone. Mice, fasted for 20 hr, were injected intraperitoneally with 15 μ g corticosterone/kg or 0.75 IU insulin/kg. The number of mice is given in parentheses.

TABLE III
THE EFFECT OF AGE ON TAT LEVELS (20 HR FAST)

Age	TAT/DNA*	Range	No. of mice
<i>months</i>			
5	$4.5 \pm 0.5 \times 10^{-2}$	3.5–4.8	5
16	$2.6 \pm 0.3 \times 10^{-2}$	1.4–3.2	5
26	$2.6 \pm 0.2 \times 10^{-2}$	1.8–3.0	5

* Mean \pm SEM.

The above results appear to be at variance with the observations of Gregerman (1959), who did not find age differences in the TAT activity per unit

dried liver of 12–13- and 24–26-month old rats before or 4 hr after induction by hydrocortisone. However, we consider the 12–13-month old rats in Gregerman's study to be more closely equivalent to the 16-month old mice in our study than to our 4–9-month old mice; Gregerman did not measure TAT levels of rats younger than 12 months. Further consideration is given to the age-related changes in fasting TAT levels in the Appendix.

It is important to note that the lowering of fasted TAT levels occurs at an age sometime between 9 and 16 months, well before the age at which the rate of mortality increases (see Figs. 1–3); we consider this age change to be a late aspect of maturation. However, the delayed induction of TAT during cold stress appears to occur during the period of rapidly increasing mortality, sometime between 16 and 26 months (see Figs. 1–3); hence, this age change may be designated as an aspect of senescence, as defined in the Materials and Methods section of this paper.

Changes in the levels of other enzymes have been described in the livers of ageing C57B1/6J mice. For instance, glucose-6-phosphatase activity is slightly greater at 4–6 months than thereafter; no changes occur from 9–24 months (Zorzoli, 1962). In contrast, alkaline phosphatase activity is nearly constant from 2–18 months; increases of about 40% are observed after 20 months (Zorzoli, 1955). Complex fluctuations in the activity of isocitric dehydrogenase after the age of 19 months are also reported by Lang and Acree (1969). A survey of the literature on enzyme levels in various tissues during ageing has indicated that the direction and extent of change may be different for each enzyme and tissue at any stage of life; in general, changes in enzyme levels during ageing are *histospecific* (Finch, 1969).

C. Adrenal Cell Function (*Studies of Corticosterone Secretion*)

Factors secreted by the adrenal gland were shown by us to be necessary for the induction of TAT in young mice during exposure to cold (Finch et al., 1969). Adrenal corticosteroids are known to be important for the maintenance of body temperature in young rats during exposure to cold (e.g. Roos, 1943) and glucocorticoids in particular are known to stimulate the induction of TAT (Lin and Knox, 1957; Goldstein et al., 1962). As the experiments described in the present paper have indicated age-related changes in both the regulation of body temperature and TAT, we therefore examined the ability of the ageing mouse to produce corticosterone, a glucogenic steroid of the adrenal cortex secreted during exposure to cold (Kolthoff et al., 1963).

The time course of corticosterone secretion (Fig. 8) is closely similar in young adult (6 months old) and senescent mice (27 months old). The average increase of plasma corticosterone is slightly but consistently less in senes-

cent mice, although this age-related difference is not highly significant ($P > 0.05$). No age differences in basal levels of corticosterone were found, in agreement with the observations of others, e.g. Rapaport et al., 1964; Grad et al., 1967. In another study, differences in the increase of plasma corticosterone after the stress of ether inhalation or of Nembutal injections in 14 or 24 month old rats have only marginal statistical significance (Rapaport et al., 1964).

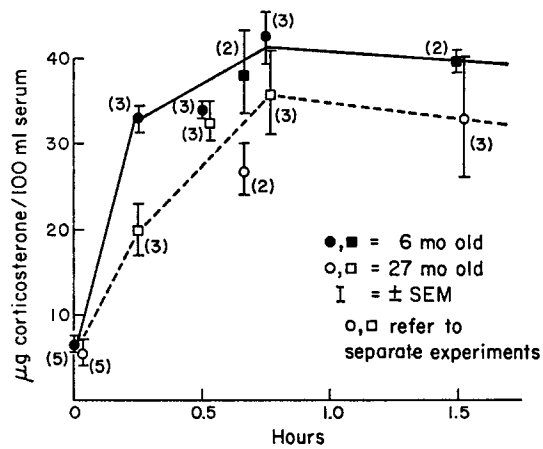


FIGURE 8. The effect of ageing on the levels of serum corticosterone during cold stress (9–10°C). Mice were fasted for 20 hr. The procedure for corticosterone assay is described in Materials and Methods. The number of mice is given in parentheses.

As corticosterone secretion is known to depend on new protein synthesis in the adrenal cortex (Ferguson, 1963), it can be surmised that a general impairment of biosynthetic activities does not occur in the adrenal cortex during ageing. The rapid course of corticosterone secretion well within the period of delayed induction of TAT during exposure to cold implies that the level of corticosterone secretion that we observed is not a quantitatively sufficient stimulus for the induction of TAT in senescent mice during exposure to cold. Finally, it is evident that the difficulty ageing mice have in maintaining their body temperature during exposure to cold cannot be ascribed to a defect of corticosterone secretion.

D. Mortality during Prolonged Exposure to Cold

Senescence was found to markedly decrease the survival of fasted mice during exposure to cold at 9–10°C (Fig. 9). The severity of the cold was observed to be an important variable, as age differences in survival were not observed at 2°C (Fig. 10).

The above results agree with observations on ageing mice (Grad and Kral, 1957) and rats (Trujillo et al., 1962) during prolonged exposure to cold with food and water available ad lib. As old age does not affect the survival of

rats during a prolonged fast (Jakubczak, 1967) and as 8-month old C57B1/6J males can survive 120 hr without food (Finch, unpublished observations), it is therefore unlikely that the increased mortality of fasting, ageing mice during a 48 hour exposure to cold is the simple consequence of inanition.

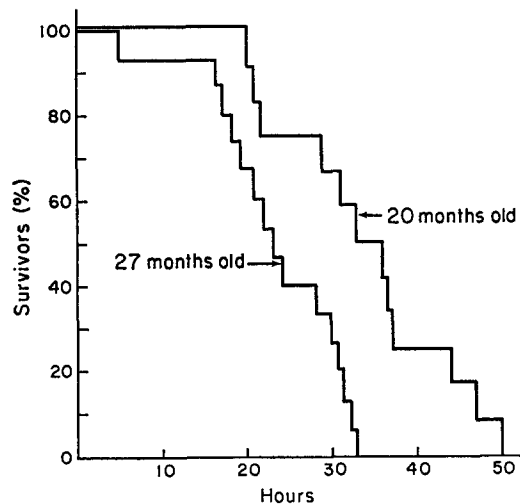


FIGURE 9. The effect of age on survival at 9–10°C. Mice were fasted for 20 hr before exposure to cold. The number of mice surviving at any time was recorded as the number of mice which had not collapsed (lost their balance, lying on their sides, etc.). 16 mice aged 27 months and 13 mice aged 20 months.

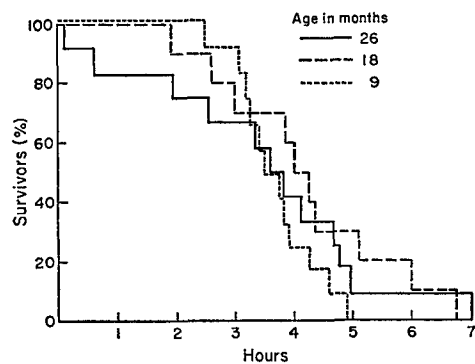


FIGURE 10. The effect of age on survival at 2°C. Experiment conducted as described in Fig. 9. 12 mice aged 26 months, 10 mice aged 18 months, and 12 mice aged 9 months.

The decreased ability of ageing mammals to survive stressful experiences (e.g. the decreased survival of ageing mice during the stress of exposure to cold described above) may play an important role in the increase of disease and mortality during ageing, for it is well-known that various stresses may themselves cause or precipitate disease (Selye, 1946; Wolff, 1968). Moreover, ageing mammals would appear to be less able to withstand the stress which disease itself imposes on the body. In this way, a change in the regulation of body processes could set an upper limit to longevity.

DISCUSSION

Thermoregulation is markedly affected by age in the C57B1/6J male mouse. In contrast to fasted young mice, it was found that fasted senescent mice were strikingly unable to maintain normal body temperatures during exposure to cold. Studies of the liver and adrenal cortex revealed a selective effect of ageing on certain cell activities which are concerned with gluconeogenesis [viz., the induction of hepatic tyrosine aminotransferase (TAT) and the secretion of adrenal corticosterone]. The liver and the adrenal cortex are well-known for their role in regulating metabolic interconversions (e.g. gluconeogenesis) which are the basis for thermogenesis.

The induction of hepatic TAT was studied as a function of age. It was established that induction of TAT during exposure to cold in fasted senescent mice was dependent on gene activity, as previously shown by us for young mice (Finch et al., 1969). However, in contrast to the linear increase of TAT in fasted young mice upon exposure to cold (Finch et al., 1969), we repeatedly observed a marked delay (up to 2 hr) in the induction of TAT in senescent mice. After the termination of the delay, the rate of increase of TAT activity was closely similar to that prevailing in young mice. It was also observed that direct challenge of the liver with insulin or corticosterone resulted in a rapid induction of TAT in both young and senescent mice. As the induction of TAT by these hormones is known to be dependent on gene activity, it can be concluded that senescence has not impaired the *potential* for rapid, gene-mediated cellular responses. This result provides an example of a change in a gene-dependent cell process during senescence (delayed TAT induction during exposure to cold) which is not due to a change in the potential of the genome for responding when sufficient stimulæ are applied (direct challenge with exogenous hormones).

The particular stage or stages of TAT induction which are delayed in senescent mice during the stress of exposure to cold cannot be identified at present. As the rate of increase of TAT activity after the end of the delay in senescent mice is closely similar to that prevailing in younger mice during exposure to cold and as increases of TAT activity during hormonal induction have been shown to result in a corresponding increase in the rate of synthesis of the polypeptide chains of TAT (Kenney, 1962), it can therefore be deduced that age does not affect the rate of TAT synthesis once it is initiated. The absence of changes during ageing in the rate of hepatic protein synthesis is also indicated by experiments on the uptake of radioactive amino acids into whole liver proteins and microsomal proteins (Beauchene et al., 1967) and on the turnover of liver cytochrome *c* (Fletcher and Sanadi, 1961). Future experiments may reveal rate changes in the generation of the endogenous

stimulae which trigger TAT induction in the liver, penetration of these stimulae into the liver cells, consequent response of the liver cell genome (including transcription), or the flow of information from the nucleus to the cytoplasm.

The demonstrated sensitivity of the regulation of liver TAT to the state of the body (e.g. to many hormonal and nutritional factors, surveyed in Table IV), and the ability of senescent mice to rapidly induce TAT without a delay in the response to injections of insulin or corticosterone, together strongly imply that the delayed TAT induction in senescent mice during exposure to cold results secondarily from the response of liver cells to a primary *extrahepatic* age change in humoral factors of the internal environment. According to this concept, the liver merely reflects age changes which originate elsewhere in the body.

TABLE IV
FACTORS INFLUENCING TAT LEVELS

TAT increased by	TAT decreased by
Glucocorticoids (Finch et al., 1969; Goldstein et al., 1962; Lin and Knox, 1957)	Growth hormone (Kenney, 1967)
Epinephrine (Wicks, 1968)	Protein-free diet (Watanabe et al., 1968)
Insulin (Hager and Kenney, 1968)	
Glucagon (Hager and Kenney, 1968)	
High dietary protein (Watanabe et al., 1968)	

The activities of the adrenal gland, shown to be necessary for TAT induction during exposure to cold in young fasted mice (Finch et al., 1969), do not seem to be significantly altered during ageing as judged by the secretion of corticosterone, a glucocorticoid.

In other experiments with young mice, we have shown that cold does not elicit the induction of TAT during a refractory period which persists for at least 12 hr after the beginning of the most recent feeding: the capacity for induction of TAT during cold is regained within 20 hr after feeding. However, direct challenge of the liver in young mice (not exposed to cold) with injected insulin during this refractory period (12 hr after feeding) resulted in a vigorous induction of TAT (Finch et al., 1969). This implies that the stimulae which mediate the induction of TAT during exposure to cold are either not released during the postprandial refractory period *or* their effect on the liver is inhibited. Possibly, the delay in TAT induction during exposure to cold in senescent mice represents an extension of the postprandial refractory period, which is known to end earlier than 20 hr after feeding in young mice.⁶ Al-

⁶ The fast which preceded these experiments was begun at noon of the preceding day. As mice of all ages in our colony normally cease eating before 7 a.m., it can be estimated that all mice were fasted at least 25 hr before being subjected to cold. The postprandial period during which young mice are refractory to the induction of TAT by exposure to cold is known to end within 20 hr after the *beginning* of the most recent period of feeding (Finch et al., 1969).

ternatively, the reduced internal temperature of senescent mice during exposure to cold may affect some temperature-sensitive step in the complex chain of events which culminates in the increase of TAT activity.

In conclusion, we believe that the selective age-related changes in liver cell activities (the lower TAT levels and the delayed induction of TAT during exposure to cold) are best interpreted as a reflection of age-related changes in humoral factors of the internal environment, rather than as a result of accumulated random genomic damage in the liver. Supporting this interpretation is the unimpaired ability of the liver to regenerate during senescence (Bucher and Glinos, 1950; Bourlière and Molimard, 1957). Age-related changes in the humoral factors of the internal environment are indicated by the experiment of Horton (1967) in which the slowed regrowth of hair in the skin of senescent mice appeared to be restored to the rate of hair growth in young mice upon transplantation. Here age-related changes in hair follicles are clearly implied as the result of a primary age change in humoral factors. Although the extent to which humoral factors can account for age-related changes in cell activities of mammals is currently unknown, it is difficult to imagine how random damage to the genome of cells throughout the body could result in the selective patterns of age change described in the above experiments and in the data of others which were considered in the Introduction. Age changes in any cell activity are selective with respect to (a) the cell type affected and (b) the age at which the effect becomes manifest (reviewed in Finch, 1969). Future studies may establish that selective changes in mammalian cell activities during ageing result from selective changes in gene activity, a response of the genome to alteration in the internal environment of the body. In the case of the Pacific salmon described in the Introduction, selective cellular age changes are the result of hyperadrenocorticism, a condition resulting from a change in regulation of the gonad-hypothalamus-pituitary-adrenal axes. If, as we suspect, the changes of TAT regulation described in the present paper are the secondary result of a primary extrahepatic age change in humoral factors of the internal environment, involvement of the neuroendocrine system, long known as the source of body temperature regulation, may be conjectured.

APPENDIX I

It is possible that the calculation of TAT activity per unit liver DNA is faulted by a large loss of functioning hepatocytes (Falzone et al., 1967) or by gross increase of connective tissue during ageing (Hinton and Williams, 1968). Five randomly selected, nonfasted mice from each of three age groups (5, 16, and 26 months old) were killed at 8 a.m. and their livers were examined for evidence of such changes.⁷ Hematoxylin- and eosin-stained sections (5 μ) were examined microscopically; the normal ap-

⁷ We are indebted to Dr. Arthur Hurvitz of the Laboratory of Comparative Pathology, at The Rockefeller University for assistance in this examination.

pearance of hepatocytes in a recently fed mouse (which had been laden with glycogen before the staining procedure according to the criteria of Ham [1965]), was observed in each case. There were no areas of necrosis such as are occasionally reported in the livers of ageing rodents (e.g. Falzone et al., 1967). There were occasional regions of slight hyperplasia of the portal bile duct epithelium. Instances of this occurred in livers of some mice from all age groups and could not, in a hypothetical case, distort the fraction of hepatocytes (presumably the site of inducible TAT) sufficiently to reduce the fasted TAT/DNA levels by 30–40% in middle aged and senescent mice. Therefore, we interpret the reduction of TAT/DNA levels, which occurs sometime after 9 months of age, as the result of a resetting of the hepatocyte TAT levels.

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