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Review Article

Biological Aging and Its Modification of Drug Activity

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THE PROCESS of zoological aging may be defined as the progressive orderly alteration in the metabolism of an animal with the passing of time, the conclusion of which is characterized by the complete and irreversible arrest of energy production or death. According to this definition, the demarcated events initiated in vertebrates at the moment of conception and continued through the differentiation of the embryo and fetus constitute as much of an integral part of the aging process as the more familiar interdependent metabolic and morphologic changes that occur in the adult as he approaches senescence. In this light, the accepted concept of aging, generally expressed as a linear decline in metabolic activity overtly manifested by a gradual loss of vigor and resistance, is inaccurate, for in actuality many higher organisms, including man, reach a period of peak vigor before the attainment of adulthood when subtle physiologic modifications commence, only to be consummated at a later date in the syndrome known as "old age." The scope of this review, therefore, encompasses the influence of biological aging on the action of drugs rather than what might be termed the gerontologic basis for the modification of drug activity.

NATURE OF BIOLOGICAL AGING

Causes

Food Deprivation.—Because it is well recognized that the aging process is a biological trait common to many unicellular as well as multicellular animals (1), attention should be directed toward a clearer understanding of the reasons for the immortality of those creatures which represent the exception. The nutritional experiments of Muggleton and Danielli (2), which dramatically emphasize the importance of dietary restriction in biotransforming ageless immortal amoebae (*A. proteus* and *discoides*) into the equivalent of age-prone mortal stemline cells, are a notable example. When the organisms were placed in a culture with an unlimited supply of Tetrahymena as food, growth proceeded in a logarithmic manner, with the life span of any clone appearing to be unlimited. However, when the amoebae were grown in the presence of a reduced supply of Tetrahymena (with other food in excess), growth and cell division became static and, after some weeks, a change in the character of the clone occurred. Upon restoration of the food supply, the life of the clone was reduced to approximately 9 months. Two types of animals arose from these studies. Type *A*, the most common form, divided periodically into two cells, only one of which was viable. In several days, a daughter cell died, but the remaining cell grew and again divided. With each subsequent division, a daughter cell died, and, after many months, both cells died. Type *B* was represented by the cell capable of dividing by equational binary fission into two viable daughter cells which continued to multiply, but at an ever-decreasing rate, until death eventually overtook them. Although the authors have attributed

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these abnormal cellular deviations to such diverse causes as waste accumulation, mutations, faulty duplication of essential amoeboid organs (*i.e.*, the centrosome), and the establishment of an alternate homeostatic organization, the underlying stimulus which precipitated the changes leading to cytomorphosis in the amoeba was the deprivation of an essential dietary factor.

Among the Metazoa, the diploblastic sea anemones and hydroids of phylum Coelenterata (3) continually replace their entire cell populations in a manner that rivals the self-rejuvenating behavior of the amoeba (1, 4–6). Brien (4), who closely observed hydras for almost 5 years, concluded, after failing to detect any changes in tentacle budding rates, that they were theoretically ageless. Hase (7) and Grosz (8), on the other hand, noted that the number of tentacles increased with age, while Vishnevs'kii (9) also found a progressive increase in the length of the maturation period for successive bud development with the passing of time. In an experiment which utilized both irradiated and nonirradiated hydras (*Hydra littoralis*), Stevenson and Buchsbaum (10) confirmed Vishnevs'kii's observations (9) by demonstrating that hydras bud more slowly with advancing age, a condition that remained unaltered, even after exposure to a single 2000-r dose of X-radiation. According to Slobodkin (11), the budding rate of green or brown hydra is a function of food availability, for an inadequate supply results in cessation of budding and diminished body size. Indeed, in the absence of food, these organisms regress to such diminutive forms that they become incapable of devouring any metazoan. Clearly, a reappraisal of the investigational value of these and other animals which occupy the lower rungs of the evolutionary ladder should be made in the future, if only to ascertain why cellular simplicity fosters nonsenescence, while protoplasmic complexity nurtures senescence.

Irradiation.—Unlike the great degree of radioresistance exhibited by differentiating hydras in the presence of sublethal X-radiation (10), the irradiation of higher animals both noticeably affects and, conversely, is affected by age. The classic studies of Russ and Scott (12) and Henshaw (13) have shown that exposure of young rats and mice to whole-body γ- (radium) or X-irradiation caused a retardation of the growth rate and a shortening of the life span, the latter having a direct relationship to the daily X-ray dose. The protection afforded by prophylactically administered cysteine (but not cystine) and glutathione in diminishing the sensitivity of rats and mice to lethal amounts of X-rays delivered at high or low dose rates has been demonstrated by Patt *et al.* (14, 15) and Barron (16). Also, the destructive action of ionizing radiation on glutathione has been noted by Hammett (17) and Woodward (18). Barron *et al.* (19), investigating the latter phenomenon, observed that small doses of X-rays inactivated sulfhydryl enzymes *in vitro* by oxidation of the SH-groups (reversible inhibition), while large doses denatured the protein moiety (irreversible inhibition). One might be tempted to conclude from the foregoing experiments that glutathione, a normal cell constituent, and other sulfhydryl compounds could be regarded as being limited antisenescent agents in so far as they are able to prevent a drastic shortening of the life span induced by ionizing radiation in certain species. However, in common with a large number of other types of antiradiation drugs, such as *p*-aminopropiophenone (20), nitrites (21), and cyanates (22), SH-compounds apparently owe their antidotal properties to the production of hypoxia which, in turn, reduces cellular activity (23). Therefore, attractive as the hypothesis may be, one cannot speculate that the mere replacement of certain essential SH-constituents which are known to decrease with age (24) could halt the encroachment of senescence in the higher vertebrates, for the attainment of an adequate degree of protection against radiation damage by cysteine and glutathione necessitates the administration of massive, nonphysiologic, near-toxic doses immediately prior to irradiation (23). A more fruitful approach to the problem would be the acquisition of a basic understanding of the reasons for enzymatic depletion coincident with age.

The influence of age on radiation sensitivity of mice and rats has been studied by Zirkle *et al.* (25), Abrams (26), Hursh and Casarett (27), Kohn and Kallman (28), and Sacher (29, 30). These investigators are in general agreement that radiosensitivity changes due to aging are slight during the first half of the adult life span. Sacher (30), in a pilot study of the age dependence of acute radiosensitivity, exposed female mice to 100 r of X-radiation daily (5 days per week) throughout their lives and concluded that sensitivity increased rapidly during the second half of adulthood. He also postulated that injury sustained by radiation was proportional to the dose, that recovery was proportional to the amount of radiation damage, that all kinds of radiation injury summated with age effects, and, finally, that injury due to aging accumulated as a linear function of age. Consequently, the curve of LD_{50} *versus* age should be approximately linear during the first half of life and concave upward

throughout adulthood. Jennings (31), noting the importance of adequate protein intake in decreasing the sensitivity of rats to total body irradiation, has stressed the value of maintaining nutritional constancy in age-dependence radiation studies. Leaf and Neuberger (32) reported that the concentration of liver GSH was reduced during protein deprivation, while Edwards and Westerfeld (33) showed that a protein-free diet reduced liver GSH in rats to approximately 40% of the normal value in several days without altering blood concentrations. Because shielding of the liver *in situ* has been demonstrated to protect 73% of a group of rats against a dose of X-rays that was 100% lethal after 15 days to the controls (34), the pre-eminence of the role of hepatic GSH in relation to radiosensitivity responses of the rodent becomes evident.

Evolution.—Although evolution is not regarded as a direct cause of aging, the differences in longevity among the various groups of the earth's fauna have arisen as an indirect consequence of it, for each species has a life span contingent not only upon its genetic heritage but also upon the evolved limitations of its physiological processes. The creation of species was undoubtedly engendered eons ago in the reaction of primordial protoplasm to the hostility arising from a changing environment. The temporal and environmental interrelationships as circumstances favorable for the production of new forms through natural selection have been aptly described by Darwin in "The Origin of Species" (35): "Though Nature grants long periods of time for the work of natural selection, she does not grant an indefinite period; for as all organic beings are striving to seize on each place in the economy of nature, if any one species does not become modified and improved in a corresponding degree with its competitors, it will be exterminated."

Reposing in mute testimony to the reliability of Darwin's statement are the vestigial structures found in the bodies of contemporary *Homo sapiens*. These remnants of functional organs are reminders of periods in the distant past when great anatomical transitions occurred, *i.e.*, postural erectness and greater head mobility (associated with loss of control of ear muscles) and the transformation from an herbivorous to a carnivorous creature (causing atrophy of the terminal caecum to become the vermiform appendix) (36).

The biogenetic law of Haeckel, expounded in 1874, which summarily states that "ontogeny recapitulates phylogeny," or that during prenatal development the embryo passes through each successive adult stage of its ancestors, has been supplanted by the more accurate concept of paleogenesis which recognizes not only the tendency for the developing organism to "climb its family tree" but also the importance of "recapitulations of ontogenies" as well as adult stages (37). The life history of man and other higher organisms, arbitrarily divided into pre- and postnatal epochs, involves sequential episodes of rapid growth and differentiation (when aging is most rapid), followed by maturity and old age, or senescence (38). The life cycles of component tissues and organs reflect the aging patterns of the whole organism, although they are accomplished at different rates which are dependent upon the intrinsic character of the individual life cycles of their constituent cells. The indirect effect of evolution on aging thus becomes apparent as does the realization that paleogenesis is not confined soleyl to the period of prenatal growth but continues throughout the life of the organism. Therefore, it is understandable how such an unusual phenomenon as neuronal multiplication can occur postnatally even in the absence of mitotic figures, if one considers that these cells could arise from primitive precursors (latent ependymal cells) which, under the proper influence, multiply and become differentiated into functional neurons. Altman (39), investigating brain cell proliferation autoradiographically by injecting thymidine-H^3 in rats and cats, has suggested this possibility after finding that glia cells actually proliferate in both species.

Structural and Metabolic Changes

The preliminary discussion of several interesting experimental approaches to the study of age causation in animals of low and high phylogenic orders should alert the reader to an awareness of the difficulties encountered in extracting from the literature generalizations pertaining to the influence of aging on drug activity, for increasing cellular complexity may parallel a greater unpredictability of response, while increasing age at the time of insult may evoke effects comparable to those that would have occurred had the agent been given at the other age extreme. Therefore, it is essential that the reviewer examine in some detail the microscopic, macroscopic, and metabolic alterations accompanying the aging process in higher organisms before arriving at definitive conclusions with respect to how these changes modify drug action.

Pigment Deposition.—The relationship of age to the accumulation of intracellular pigment is a phenomenon whose biochemical significance remains unknown. Mildvan and

Strehler (40) observed a linear deposition with age of a yellow-orange fluorescent particulate component in human myocardium approximating from 6 to 10% of the cell volume at advanced age. In studies of the quantitative changes occurring in the cerebral cortex, Brody (41) examined pigment deposition within cortical nerve cells in order to compare the pigment content of cells within different cortical areas in a normal human series ranging from newborn to age 95. The precentral, postcentral, and superior temporal gyri followed a similar pattern in regard to the number of cells with pigment granules and the amount of pigmentation in each cell. In all areas studied, a direct relationship existed between the number of cells with granules and the age of the specimen, *i.e.*, number of cells affected and degree of involvement increased with age. Sinex (42), in a comprehensive review of the biochemical aspects of aging, cited the mounting evidence for pigment accumulation in certain structures having been formed from autooxidized lipid, implying that autooxidation may be a characteristic feature of senescent tissues where the reaction presumably takes place by a free radical mechanism entailing the formation of peroxides and carbon and oxygen radicals. If this hypothesis proves to be correct, a speeding-up of the aging process with trace metals, hematin, hydrogen peroxide, and oxygen, or a slowing-down with specific antioxidants should be effected (42). Widespread pigment deposition, extracellularly and intracellularly, in the aging body could modify drug activity by interfering with or displacing enzyme systems, altering membrane permeabilities, or by physically decreasing the number of active receptor sites.

Connective Tissue Changes.—The internal environment of the whole organism is upset during the later stages of aging as gross transformations in the character and arrangement of various kinds of connective tissue disrupt nutritional (anabolic) and excretory (catabolic) pathways (43). Shanklin and D'Angelo (44), studying reticular fiber changes in a series of 100 human pituitaries, newborn to 96 years, observed that numerous thin fibers in the anterior lobe of the neonate became even more numerous and thicker with aging. Similarly, the sparse reticular fibers in the immature neurohypophysis gradually extended from the walls of large blood vessels into the surrounding area over a period of 20 years. After age 40, a considerable increase in fiber distribution was noted and, in old age, the original bundles had disappeared, leaving behind only a meshwork of widely scattered fibers. Comparable increases in fibrous connective tissue in other parts of the body have been described (43).

The amorphous/fibrous intercellular substance ratio gradually changes from the jellylike consistency of the fetus (high amorphous/low fibrous intercellular content) to the tough, leatherylike texture of the senescent individual (low amorphous/high fibrous content). Aged tissues contain such minute amounts of amorphous intercellular substance that dyes diffuse through them more readily than they do in younger tissues, the free passage of dyes being impeded by the amorphous substance present (45). In the young, elastic fibers are free of mineralization; in the aged, fraying, fragmentation, and the development of an affinity for calcium salts occurs (46). The average size of collagenic bundles and individual fibers increases with age, and the substance becomes more basophilic (45).

It is well known that skeletal growth and the growth remodelling which accompanies it ceases in man at age 17–20; yet internal remodelling, defined as "the process of replacement of primary appositional bone with Haversian bone occurring within the periosteal-endosteal envelope," undergoes a major change at age 35 and increases thereafter (47, 48). Measurements of the surface area of Howship's lacunae in rib diaphyseal cortex for 137 normal individuals of both sexes, aged 1 month to 84 years, revealed that in childhood internal remodelling resorption is at a maximum and declines to the fourth decade, after which it rises. Accordingly, throughout life the activity of osteoblasts is constantly changing, while osteoclastic activity follows alterations in a similar degree and fashion (48). In the guinea pig, Myers *et al.* (49) observed a steep decline in the osteoclastic population with increasing age at the metaphyseal aspects of the mandibular condyles between birth and 24 weeks. Basmajian (50) examined the depressions or pits on the calvarium caused by arachnoid granulations (of Pacchioni which are absent during the first decade and increase in number with advancing age) in 148 subjects and found that, although wide variations in the size of the depressions occurred from individual to individual in any age group, they generally became progressively larger with increasing age. The author concluded, however, that pit size cannot be used as an accurate criterion of age in the medicolegal sense.

In mice, the rate of skeletal growth is contingent upon the strain, sex, and, in certain strains, the sexual status of the female (51). Breeding accelerates skeletal aging; the onset and progress of age changes were considerably

delayed in virgin females in contrast to breeding females, and males. In the latter, aging of the skeleton started more slowly than in females but eventually proceeded more rapidly than in either virgin or breeding females (52). According to Lexer (53), aging causes the bone vasculature to become thinner and less numerous. Mineralization, which increases in aging bone at the expense of organic matter, results in greater fragility, reduced permeability, and more compactness (54).

Hematological Changes.—Red blood cell and hemoglobin variations at different ages have been described for the rat, rabbit, pig, cat (55, 56), and man (57, 58). In all of these species, the erythrocyte count and hemoglobin concentration were high at birth, then decreased shortly after birth due to regression of erythrocyte size and number, and again at maturity gradually increased to adulthood, at which time the erythrocyte size remained constant.

Trevorrow *et al.* (59) studied plasma protein changes in 547 healthy persons, aged 1 day to 39 years, and observed that each of the protein fractions changed with age in early infancy and childhood. During the first month after birth, plasma albumin was low (3.79 Gm./100 ml.), but a distinct rise with increasing age soon became apparent. Between 6 months and 1 year, the adult level was attained. From birth to the end of the first week, plasma globulin was only 1.66 Gm./100 ml.; yet between the first and fifth week, the value fell to 1.31 Gm./100 ml. However, at 6 months, a gradual rise occurred to age 4, when the adult level was reached, whereupon no significant changes were detected during the next 35 years. Plasma fibrinogen showed a higher mean value and a greater variation at birth than in older age groups (0.21 Gm./100 ml.). In premature, newborn, and normal infants, Darrow and Cary (60) measured the serum concentration of total protein and found it relatively low in all infants compared to that of the adult; the decrease was due mainly to the depressed globulin fractions. Although the lowest globulin levels were determined in premature infants, the postmortem serum of small fetuses had essentially the same albumin/globulin ratio as that of full-term infants. It was suggested that the low globulin levels in infants are indicative of a lack of the usual stimulus that initiates production of the plasma protein in adults.

The age dependence of the blood sugar level was first noted by Brown (61), who obtained readings on 12 infants under 2 weeks of age by MacLean's method. Blood glucose values ranged from 0.072–0.097 Gm./100 ml., with an average of 0.087 Gm. Infants older than 6 weeks had a range of 0.086–0.116 Gm./100 ml., with a mean of 0.106 Gm. In a similar investigation, Norval *et al.* (62) determined blood glucose levels in 51 normal newborn infants and found an average increase of 2.8 mg./100 ml. per day during the first 6 days of life. Gillum *et al.* (63) evaluated at 5-year intervals the postprandial blood sugar levels of 430 normal men and women over 50 years of age and discovered little variation with either age or sex. The over-all mean was 101 mg./100 ml., which was significantly higher than means for young adults. Interestingly, men over 65 years old, confined to county homes, had significantly lower blood sugar levels than those who lived in their own homes. To introduce an unusual comparison of neonate and adult human (omniverous) blood sugar levels, on the one hand, with those of a strictly herbivorous creature—the sheep—on the other, the studies of Mann and Zarrow (64) should be mentioned. These investigators found that the average nonfasting blood glucose concentration in the ram and ewe was 0.067 Gm./100 ml., with a range of 0.056–0.075 Gm./100 ml. Approximately half of the values were between 0.070 and 0.075 Gm./100 ml. In nursing lambs, an average blood sugar level of 0.126 Gm./100 ml. was obtained, with a range of 0.063–0.225 Gm./100 ml. The authors stressed that the wide variations observed in the newborn were probably because blood was obtained at different intervals after nursing which also would account for the unusually high glucose levels. The relatively depressed glucose levels of adult animals, compared with those of other mammals, may reflect a deficiency in the endocrine capability of the pituitary-adrenal axis (65).

Sedimentation rates increased with advancing age in men and women over 50 years old and also were influenced by sex, *i.e.*, higher for women (66).

Age has an effect on the blood concentrations of certain vitamins. The total serum vitamin B_{12} concentration determined in 528 normal individuals showed a trend toward a lower serum level in aged than in younger groups (67). The total circulating vitamin B_{12} in man varied from patient to patient at all age levels, but there was a general trend toward lower levels in advanced age. Decreasing concentrations of ascorbic acid in serum (except for girls over 14 years) and decreasing intakes of ascorbic acid per kilogram of body weight were associated with increasing

age in a nutritional study conducted on 650 Iowan boys and girls (68).

Cardiovascular Changes.—Waugh *et al.* (69) suggest that the common vascular alterations of atherosclerosis, calcification, and hypertension, the consequences of aging in man, are less likely to develop in herbivores. Chemical analyses of the aortas from 44 female rabbits in two age groups (3 and 46 months), corresponding to human childhood and middle-age, indicated an elevation of water, sodium, calcium, nonlipid phosphorus, and possibly of the cholesterol content of the vessel wall in the older group. The authors imply that the generally lower levels of total lipid and cholesterol in the rabbit aorta, compared with that of man, may account at least in part for the freedom of certain herbivores from spontaneous atherosclerosis.

In mice, a loss of elasticity, fibrosis, and calcification and an increase in fat content take place in the life history of elastic arteries. A widening of the aorta due to the accumulation of interlamellar components occurs in the mouse before age 20 days and remains approximately the same size thereafter (70).

Apart from a linear deposition of pigment with age (40), the human myocardium appears immune to the inroads of time (71), although distinct changes in cardiac output ensue with advancing age. Brandfonbrener *et al.* (72) employing an indicator dilution technique in 67 normal human males, aged 19 to 86, discovered that the relationship between cardiac output and age was significant, a reduction of about 1% occurring per year. Landowne *et al.* (73) noted that, although systolic pressure rose, the apparent increase in mean brachial pressure with age was insignificant. However, the total vascular resistance of the greater circulation, measured by the ratio of pressure to flow, increased with age.

Ratcliffe and Cronin (74), during a 25-year study of the frequency of arteriosclerosis in captive wild animals at the Philadelphia Zoo, observed an increase in both birds and mammals independent of age and diet. Because atherosclerotic disease of the coronaries is the leading cause of death in the United States at the present time (75), it is pertinent to mention that anthropoid apes and man (grouped together as Anthropoidea) are the only mammals to present important systematic fibrous changes of the coronary intima after puberty, according to the observations of Vastesaeger and Delcourt (76). In studying the natural history of atherosclerosis in captive and free wild animals, these authors concluded, after examining the cholesterol content and other biochemical changes in coronary arteries and aortas, that spontaneous atherosclerosis always develops in arteries impaired by intimal sclerosis and that the latter condition sets the stage for the subsequent evolvement of spontaneous atherogenesis. Also, the species variability in the time of onset and degree of vascular pathology is illustrated dramatically by the striking similarity between the coronary arterial lesions of a wild pig which died in captivity of old age and those of a 5-year-old gorilla.

Cardiac failure in the aged is invariably associated with a significant increase in heart weight, which in the absence of valvular disease, has been equated with prior hypertension (77). In dogs, aging increases the heart weight/body weight ratio (78).

Dizziness is more common in persons over 65 years old than in those of any other age group because of myocardial deterioration, atherosclerosis, or failure of the cardiac reflex to respond to positional changes (79).

Respiratory Changes.—Young mammals are more resistant to anoxia and asphyxiation than adults because during prenatal development the capacity to utilize anaerobic energy sources arises and, after birth, persists for a short time (80–82). Thus, after ligation of cerebral vessels or decapitation, the head of a newborn rat or dog gasps 40 to 80 times during a period of 30–60 min., while the head of a half-grown or mature animal ceases gasping within several seconds (83). The remarkable survival of the respiratory mechanism in neonates is neither unique nor without precedence, for other physiological processes, such as the spinal (trunk) reflexes, pupillary responses, and cardiac activity, are similarly affected (84). Undoubtedly, the increased erythrocyte count and hemoglobin concentration present in mammals at birth (55–58) contribute in some degree to the anoxic resistance by facilitating oxygen transport.

Alterations in pulmonary function with advancing age were first recognized by Hutchinson (85), who noted a decrease in the vital capacity with age. Cander and Blumenthal (86) observed a moderate decrease in ventilation efficiency of the lungs due to a progressive increase in the number of alveoli supplied with less than an optimal quantity of pulmonary capillary blood flow, thereby giving rise not only to a reduction in vital but also in total lung capacity.

Gastrointestinal Changes.—Aging of the gastrointestinal tract results in atrophy and deterioration of the colonic musculature, thinning of intestinal walls, and a reduction of peristaltic activity (87). After age 50, pear-

shaped diverticula form and, with poor elimination, become filled with fecal masses which lead to irritation, infection, and diverticulitis (87). The incidence of polyps of the colon is relatively high in children, but as puberty is approached, it decreases. During adolescence, the frequency gradually rises until it reaches a maximum among the 60–70 year age group (88).

According to a study conducted by Lee (89), concerning the influence of sex and age on the incidence of appendicitis and of related fatalities in children and young adults, the highest frequency of the former occurred at age 12 in boys and age 10 in girls. Although the author found that appendicitis was uncommon in children under 5 years, a large proportion of the cases was complicated by peritonitis; consequently, the death rate was unusually high. The peak incidence for cases of this nature appeared in children between 4 and 5 years old.

Friedman (90) cautions that the ulcer patient who has hemorrhage or perforation after age 50 should be treated with definitive surgery when conditions permit. However, operative procedures become mandatory if the hemorrhage does not cease within the first 12 hr.

Organ Changes.—*Brain and Peripheral Nerves.*—Pigment accumulation, fatty degeneration, shrinkage, atrophy, and loss of nerve cells occur in various areas of the brain and spinal cord with age (91). The glycogen content in different parts of the cat and dog brain was determined at various ages by Chesler and Himwich (92) who found that the concentration increased with age in the newest phyletic parts—the cerebral cortex and the caudate nucleus. On the other hand, the percentage of glycogen in the oldest areas—the cerebellum, medulla, and spinal cord—fell progressively with age. Mannell and Rossiter (93), investigating the biochemical aspects of peripheral nerves, discovered that the sciatic nerves of a group of rats 60 days old contained higher concentrations of nucleic acid and phospholipid than those of rats aged 160 days. After nerve section, an increase in the concentration of nucleic acid and a decrease in the amount of phospholipids occurred in both groups. Also, Wallerian degeneration was more rapid in the younger animals.

Liver.—Hutterer *et al.* (94), measuring the amount of hydroxyproline in the liver (a measure of collagen presence) found that less is present in infants than in adults, whereas more is present in cirrhotics. In both infant and cirrhotic livers, the ratio of soluble to total collagen was much higher than in adult controls. As an animal ages, its liver cells lose their metabolic capacity and attempt to regain some aspects of the resulting deficiencies by forming more of the active sites within the cell (95). Johnson and Albert (95) determined the nitrogen and phosphorus contents of large and small granules and cytoplasmic supernatants from the livers of mice, aged 1.5 to 21 months. Increased nitrogen contents and relatively smaller increases in phosphorus contents were detected in the older animals. The cell fractions of the older group differed chemically from those in the younger animals, indicated by a decreased P/N ratio.

Thymus.—Of all the endocrines, the thymus appears to be the one most profoundly influenced by age. At birth, the human thymus weighs approximately 15 Gm.; at puberty, 35 Gm. During the period from puberty to late middle-age, it involutes progressively until at age 60 its weight is about 15 Gm. (96). Nakakuki and Nishizuka (97), studying age changes in two high-leukemia strains of mice, ascertained that the age of onset of thymic involution was well correlated with the time of sexual maturity. It is well known that castration delays thymic involution, while the administration of sex steroids and gonadotropins promotes atrophy in rodents (98). The full significance of these age–sex–hormone interrelationships, however, is not yet understood.

Adrenals.—Cooper (99) found in humans that as age advanced, a thickening of the capsule took place, and after middle life, an increase in connective tissue appeared in the zona reticularis and medulla. Dribben and Wolfe (100) examined the connective tissue of the adrenal glands from 80 virgin female rats, aged 10–884 days, and detected similar age changes in the zona reticularis. A coarsening of reticular fibrils and an increased density of the reticular meshwork were evident with age. In 90-day-old rats, collagenous fibers first appeared and gradually increased in amount and in the thickness of the individual fiber. Also, a slow and progressive thickening of reticular fibers was noted in the medulla. The adrenal cortex of the guinea pig showed the greatest mitotic activity during the fetal period and slowly diminished with age (101). In elderly men, the adrenal cortex showed fibrosis and a decrease in the number of parenchymal cells (102). In male rats, aged 17–900 days, the adrenal capsule increased in thickness and assumed a hyaline appearance with age (103). Shrinkage and degeneration of the capsule were especially pronounced in old rats. The medulla appeared less affected by cell degeneration than the cortex.

Enzyme Changes.—The enhanced sensitivity of the newborn to drugs, compared to the restrained response of the adult, could arise from hyperactivity of receptor sites, increased permeability of newly formed membranes, or from differences in their respective metabolic pathways. Fouts and Adamson (104), orienting their research toward an elucidation of the latter possibility, investigated the fate of a variety of drugs, including chlorpromazine, amphetamine, and hexobarbital, and reported the failure of liver microsomes from the newborn rabbit to metabolize any of these agents. At 2 weeks of age, low levels of enzyme activity were present, and adult levels were not attained until the animal had reached 4 weeks of age. The striking differences in enzyme activity between the newborn and the 4-week-old rabbit were presumed to be due to the presence of enzyme inhibitors which either disappeared suddenly or were overcome by some unexplained metabolic event. Roux *et al.* (105) noted that pyruvate oxidative metabolism was twice as active in the 4-day-old rabbit liver as in the newborn. Similarly, newborn mice and guinea pigs were incapable of detoxifying phenacetin, hexobarbital, and amido- and antipyrine and also were unable to form glucuronides, although this ability appeared within 8 weeks (106). Butt (107) detected a deficiency of glucuronyl transferase in the livers of human fetuses, newborn humans, and guinea pigs which subsequently was compensated for by the tenth day. Why a period of latency ensues in the development of enzyme systems during the transition from pre- to postnatal stages remains unfathomed, but one generalization can be offered. That is that rectification of enzyme deficiencies is accomplished long before the organism reaches maturity.

Hormonal Changes.—Several investigators, including Wells *et al.* (108, 109), have confirmed that fetal endocrines are quite capable of elaborating hormones. In two studies on castrated fetal rats, Wells and his co-workers virtually proved that the fetal testis can secrete androgen which stimulates the prenatal growth of such accessory reproductive organs as seminal vesicles and bulbourethral glands. The concentration of levarterenol (norepinephrine), which is much less in the newborn rat brain than in the mature animal, reaches adult levels in approximately 7 weeks (110). The production of this hormone by the fetal adrenals of certain species precedes that of epinephrine, whose appearance prior to parturition is essential for protection of the newborn from the stresses of readjustment in the outside world (111).

Bowman (112) presented evidence that the total amount of growth hormone in the pituitary gland of the rat increased greatly with age; however, the hormonal concentration in the gland remained constant. In female rats, Solomon and Greep (113) noted that the growth hormone concentration in the whole pituitary fluctuated within a small range with age.

The production of estrogens and corticosteroids remained fairly constant in man with advancing age, while androgen elaboration declined markedly (114). The relative increase in corticosteroid activity with age favors the breakdown of body tissue which contributes to diminished muscle volume and strength.

Modification of Drug Activity

Dyes.—During the early developmental stages when cleavage and germ-layer formation have been accomplished, the mammalian organism undergoes a period of proliferation that marks the commencement of cellular differentiation and is further characterized by an increased susceptibility to various agents, known as teratogens, which can induce congenital malformations in the newborn, if not death in the fetus. Fortunately, this period is brief, as illustrated by one of the basic principles of experimental teratology which states, "Susceptibility to teratogenesis decreases as differentiation proceeds" (115). Wilson *et al.* (116), using single i.v. injections of trypan blue in pregnant rats, determined that the peak of teratogenic activity fell on the eighth day of gestation and that days 7 to 9 spanned the entire period of embryonic responsiveness. A preponderance of defects developed in the central nervous system, the eye, cardiovascular system, and caudal region of the vertebral column, coinciding with the time of morphological differentiation of these primordia on the eighth and ninth days of pregnancy. In a study directed toward elucidating the structure-activity relationships of various azo dyes in producing congenital malformations in the offspring of pregnant rats, Wilson (117) administered a number of chemically related dyes and found that only three—azo blue, trypan blue, and Evans blue—were active in this respect. The author attributed the teratogenic activity of these compounds to the mutual possession of a diazotized *o*-tolidine grouping. The influence of age on the response of bone tissue to alizarin dyes was studied by Ercoli and Lewis (118) in mice. Single i.v. injections of 20–30 mg./Kg. of the dye caused bone coloration in growing animals, but adults required two to three times this dosage to elicit comparable ef-

fects. Apparently, the higher dose required for coloration in adult mice is dependent upon the anatomical and physical rather than on the chemical conditions of bone. In mature animals, less coloration was produced by the s.c. administration of the dye than by i.v. injection due to connective tissue changes in the older animals.

Autonomic Drugs.—The s.c. administration of epinephrine (0.02 mg./100 Gm.) in male rats, 2–28 months old, caused an average maximum increase in oxygen consumption of 34.5% within 45 min. after injection above the preliminary basal level. In older rats, the maximum increase in oxygen consumption was less. The maximum increase in the respiratory quotient was practically the same and reached at the same time in all age groups, but the return to normal was delayed in proportion to increasing age. The authors suggest that the delay in the s.c. absorption of epinephrine proportional to age is the possible cause (119).

At the extremes of age, in infancy and senescence, atropine fails to accelerate the heart rate in man (120), presumably because of immaturity of the intrinsic cardiac nervous system (vagi) in the former and of deterioration in the latter. Truex *et al.* (121), investigating the vagal influence on cardiac function in young puppies, employed electrocardiographic data to correlate animal heart rate with age, weight, anesthesia, and vagal stimulation and found that the heart rate was more closely related to the heart weight than to either body weight or age. Their results indicated an immaturity of the intrinsic cardiac nervous system, although the vagi were excitable at all ages.

In kittens and puppies maintained at an environmental temperature of 30°, hexamethonium, administered s.c. at a dosage level of 10 mg./Kg., caused a rapid and pronounced fall in oxygen consumption and body temperature (122). Older and heavier kittens responded less dramatically, the effect having disappeared by 52 days. The explanation for the metabolic depression produced by hexamethonium is that it prevents norepinephrine release, thereby preventing the thermogenic activity of the amine.

Central Nervous System Depressants.—The neonate is extremely susceptible to barbiturate depression because of hepatic enzyme deficiencies in the fetus and newborn infant which prevent the detoxication of these compounds (104, 106). Comparable doses by weight of barbital produced 360 min. of sleep in 1-day-old mice, over 100 min. in 7-day-old mice, and less than 5 min. in adults (123). Streicher and Garbus (124) demonstrated age and sex differences in the recovery time of rats anesthetized with hexobarbital which were explained on the basis of sex hormone levels.

Sajner (125), studying ether consumption of juvenile and adult mice, found that the younger the individual, the greater the resistance to the anesthetic per unit weight. Buchsbaum and Buchsbaum (126) subjected mice, aged 2 weeks to 35 months, to diethyl ether inhalation and discovered that old animals took longer to fall asleep than young mice, and that both old and young mice required longer periods to recover than did adults 1 to 8 months old.

Courvoisier and her co-workers, during their pioneer studies of phenothiazine pharmacology, observed that chlorpromazine elicited a profound fall in body temperature in adult animals, a response that has since been noted in a wide variety of species, including man (127–131). Recently, Bagdon and Mann (132) demonstrated that the effect of chlorpromazine on the body temperature of mice was influenced by age, for the drug caused significant hyperthermia in 10-day-old and hypothermia in 35- and 38-day-old animals. Promazine, the dechlorinated analog of chlorpromazine, was shown to be qualitatively similar in its thermotropic activity to chlorpromazine, but quantitatively it was less potent than its chlorinated derivative (133). A study of the factors modifying chlorpromazine hyperthermia in young mice revealed that pilocarpine administered prior to the phenothiazine inhibited the hyperthermia, suggesting that maturation of cholinergic receptors may be implicated in the response (134).

Chesler *et al.* (135) administered lethal doses of morphine sulfate to adult rats, newborn, and pregnant rats at full term and found that the newborns were more resistant to acute respiratory failure than adults, while the fetuses were approximately equal in susceptibility to their mothers. The greater resistance of the newborn to respiratory failure, compared to the fetus and adult, was explained by the ability of the former to activate its respiratory centers anaerobically and thus make available energy released by aerobic processes.

Cytotoxic Poisons.—When fresh unbuffered solutions of sodium cyanide were injected s.c. into newborn and adult mice, marked differences between the two groups appeared (136). In the newborn, 30–70 min. elapsed before death; adults died in less than 4 min. However, if ultimate death or survival of the animal is the only factor measured, the newborn is more susceptible than the adult, *i.e.*, the LD_{50} for

newborn mice was 2.3 mg./Kg. and for adults, 5 and 3.4 mg./Kg. (male and female, respectively). The author emphasizes that because most of the current knowledge concerning resistance of the newborn to anoxia and anoxic agents has come from studies in which the persistence of movements has been measured, these results seem to call for a careful re-examination of the effects of anoxia.

It has been stated generally that in CO asphyxiation, the smaller and younger individuals with more active metabolism approach saturation more rapidly and therefore succumb sooner than large individuals (137). Four parts of CO in 1000 parts of air is considered fatal to man after 1-hr. exposure and more fatal to smaller animals such as mice. Yet in pure concentrations of chemically inert gases, the young survive exposures from three to 56 times longer. In no instance does a young animal die during 1-hr. exposure to 0.4% CO, a concentration which is considered to be lethal to man for that period.

Halogens.—Finn and Kramer (138) have shown that fluorine incorporated in a rachitogenic diet increased the life span of the rat. The average age of death of rats fed a rachitogenic diet (controls) was 65.23 days; rats fed a fluorine-supplemented diet lived 76.66 days.

In a study of the effect of age upon the deposition and retention of fluorine deposited in the rat femur, Miller and Phillips (139) found that the halogen accumulated in greater amounts in young animals than adults and that both young and mature femurs continued to concentrate fluorine progressively with age.

One day after the oral ingestion of a small dose (0.01 μc.) of an I^{131}/I^{125} mixture, the radioiodine content of the thyroid gland was similar in children and adults (about 20% of the ingested dose) as was the biological half-life of the iodine (about 90 days) (140).

Diabetogenic Agents.—The optimum alloxan dosage for the production of diabetes (mellitus) in the rat varied with age (141). Young rats tolerated 60–70 mg./Kg., which is in agreement with Mann and Stare (142), while adult animals presented similar results at dosage levels of 45–50 mg./Kg. of the drug.

Hormones.—Baker and Schairer (143) discovered that adrenocortical extract inhibits hair growth less effectively in immature than in adult rats. Their experiments showed that age modifies the tissue response to the local action of adrenocortical hormones, for when growth is retarded by the presence of excessive amounts of these substances in the tissue fluids, the action of pituitary growth hormone is antagonized. The authors theorize that young rats may produce more endogenous growth hormone than adults or that their peripheral tissues may become more responsive to it, which would explain the reason why the inhibitory activity of adrenocortical steroids is less pronounced in immature animals.

A single injection of cortisol acetate in young mice induced a wasting syndrome similar to that observed in runt disease and in the postthymectomy syndrome (144). The course of the disease was less severe if the dose was decreased, or if the animals were older at the time of injection.

SUMMARY

The evolution of complex multicellular animals from relatively simple unicellular organisms, with a concomitant change from cellular independence to cellular interdependence, may have been indirectly responsible for the onset of aging in metazoans. The histological make-up of the fetus and newborn is designed to protect against physical trauma and the simple diffusion of noxious agents, while their physiological make-up belies an increased susceptibility to certain foreign agents because of either relative or absolute enzyme deficiencies, a situation that has its counterpart in old age. The rate of aging of each organ reflects the life cycle characteristics of its constituent cells and is dependent upon genetic heritage and the evolved limitations of its physiology. For this reason, the action of drugs at either extreme of age may be altered profoundly. The extracellular and intracellular accumulation of pigment and the extensive infiltration of connective tissue throughout the body with advancing age decrease the total viability or reactivity of the body mass and therefore necessitate a corresponding reduction in drug dosage.

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Research Articles

Kinetics of Aggregation in Suspensions

Effects of Added Electrolytes on the Aggregation Rates of Latex Particles in Aqueous Ionic Surfactant Solutions

By W. I. HIGUCHI, T. O. RHEE, and D. R. FLANAGAN

Following procedures developed earlier involving the Coulter counter, the rates of aggregation of polystyrene and polyvinyltoluene particles in aqueous ionic surfactant solutions have been studied. The two surfactants were sodium lauryl sulfate and myristyl-γ-picolinium chloride. The rates were determined as a function of both the surfactant concentration and the added electrolyte concentration for different electrolytes. The results of the experiments have been examined by the Derjaguin-Verwey-Overbeek theory. While the data are qualitatively in agreement with the theory, the decrease in rates observed with increasing surfactant concentration at high salt concentrations, particularly for sodium lauryl sulfate, suggests that electrical repulsion is not the only significant factor contributing to the repulsive part of the particle–particle interaction.

SURFACE-ACTIVE agents play a major role in determining the gross stability and flow behavior of suspensions through their influence on the aggregation and deaggregation kinetics of suspension particles. In this regard the ionic surfactants have received considerable attention from many researchers (1–5). In nearly all of the significant studies, the investigators have employed the sedimentation rate and the sediment height methods to observe the gross effects of such dispersing agents on suspension behavior and have attempted to deduce the aggregation processes from such studies. While these sedimentation methods in conjunction with adsorption studies have led to considerable understanding of these systems, it is highly desirable to have more direct information on the aggregation process itself.

Recent studies with the Coulter counter (6–8) have indicated that this instrument may be suited uniquely for direct studies of aggregation behavior in aqueous media. In the present communication, it is demonstrated that the Coulter counter may be employed in quantitating the influences of surfactants and added electrolytes on particle aggregation rates.

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EXPERIMENTAL

Materials.—Uniform size latex particles were employed as the dispersed phase as described previously (7). Polystyrene (PS) latex particles of 2.0-μ diameter and polyvinyltoluene (PVT) latex particles of 2.05-μ diameter were selected[1] for these studies. The samples were purified as described previously (7) by repeated centrifugation in alcohol and water. In the present studies, prolonged storage of the purified suspensions in water often led to variable results, with the rates always lower than those obtained with the freshly purified suspensions. Thus, all of the results reported here were obtained from freshly centrifuged samples.

The two surfactants[2] studied were sodium lauryl

[1] Supplied by Dr. J. W. Vanderhoff and Mr. L. J. Lippie, Dow Chemical Co., Midland, Mich. The present PS sample differed from the 1.83-μ size sample used in the previous work (7).

[2] The authors are grateful to Dr. K. J. Mysels for supplying us with a highly purified sample of NaLS and to Dr. D. J. Lamb, The Upjohn Co., Kalamazoo, Mich., for a specially purified sample of MPC.