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AGE-RELATED PATHOLOGY AND BIOSENESCENT MARKERS IN CAPTIVE RHESUS MACAQUES

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ABSTRACT

During the past 15 years, our aging colony of rhesus monkeys, consisting of animals from 20 to 37 years of age, had an annual average population of 88.2 live monkeys and, of this population, an annual average of 13.9 monkeys died spontaneously or were terminated due to severe illness. From 1980 to 1994, a total of 175 autopsies of rhesus macaques, from 20 to 37 years of age, were performed. By cumulative autopsy data, the incidence of age-related pathology in various organs was surveyed. Major geriatric diseases such as coronary sclerosis, emphysema, degenerative joint disorders, cancer, and cerebral amyloid plaque began to develop in 10 to 40% of macaques after 20 years and the incidence significantly increased after 26 years of age. Approximately 12% of aged macaques from 20 to 30 years of age died annually due to such geriatric diseases with severe complications. The average survival rate indicated that half the population at 20 years of age died by 25 years and 73% died by 30 years of age. Less than 10% of macaques survived over 30 years. Using these aged macaques as well as other juvenile to adult monkeys in our Center, clinical opththalmological and reproductive endocrinological studies, and magnetic resonance imaging (MRI) of the brain were conducted to define bioaging markers of captive rhesus monkeys. Cataracts began to develop in 20% of rhesus monkeys at 20 to 22 years of age and the rate significantly increased after 26 years of age. Menopause occurred at 26 to 27 years of age. Multiple cerebral infarctions and iron deposits in the globus pallidus and substantia nigra were detected by MRI in the aged brains. These geriatric disorders in captive aged macaques appear to be natural aging outcomes, since the simple lifestyle of these captive animals offers minimal exposure to environmental factors. Our data will offer useful paradigms for preventive or experimental studies on age-related diseases.

KEY WORDS

Aging, Pathology, Captivity, Rhesus Macaque

INTRODUCTION

Similar to the human population, as captive nonhuman primates live longer, the incidence of age-related diseases such as cancer, arteriosclerosis, skeletal disorders, and senile plaque formation in the brain significantly increases (Lapin *et al.* 1979; Price *et al.* 1985; DeRousseau *et al.* 1986; Uno & Walker 1993; Uno *et al.* 1996). The incidence of spontaneously developed cancer in nonhuman primates has often been described as relatively uncommon compared with that in the human aged population (McClure 1980). These earlier data, however, largely depended on the low average age of animals studied in the past. Lapin *et al.* described how the incidence of malignant neoplasm substantially increased in monkeys over 20 years of age; 27% in baboons, 6.5% in macaques, and 12% in green monkeys (Lapin *et al.* 1982).

During the past 15 years, we have studied clinical and pathological aspects of age-related phenomena or diseases of rhesus monkeys in the aging colony of the Wisconsin Regional Primate Research Center. The data described here are based on multidisciplinary physiological, ophthalmological, and magnetic resonance imaging (MRI) studies, and postmortem pathological findings. The epidemiology of age-related diseases and a list of biosenescence markers were retrospectively obtained from the cumulative data of the past 15 years' studies.

The correlation between chronological age and biosenescent age in macaque and human data will be discussed on a comparative basis.

METHOD

Aging Colony of Rhesus Monkeys

In early 1950, Dr. Harlow re-established his rhesus monkey colony in the present location of Harlow Primate Laboratory, Department of Psychology, University of Wisconsin, after nearly complete loss of the previous colony by tuberculosis. Since then, most of the monkeys have lived in indoor single or small group (5 to 20) cages in the facility. In 1964, approximately 400 rhesus monkeys moved to the indoor facility of the newly built Wisconsin Regional Primate Research Center. Thereafter, the population gradually increased by breeding among the groups in both facilities and some monkeys were added from outside sources. Since the beginning, all monkeys were fed commercial food (Chimp-Cracker in earlier days and Purina Monkey Chow for over three decades) with supplemental fruit. In 1980, our Center held 1083 rhesus monkeys, and of this population 85 monkeys were 20 to 31 years of age. In

1983, the National Institute of Aging began to support the maintenance of this unique colony of aged rhesus monkeys. These aged monkeys were set aside and kept in single or double cages for studies by clinical bioaging programs and any invasive procedures or special diets were avoided. The annual population of monkeys in our aging colony has ranged from a maximum of 117 to a minimum of 47 during the 15 years; this was caused by year to year differences in the numbers of newcomers that reached the age of 20 years, and by the annual death rate of these aged monkeys. During the past 15 years, our aging colony has held an annual average of 88.2 live monkeys and an average of 13.9 monkeys have died spontaneously or been terminated due to severe illness. The proportional population of male and female monkeys in our aging colony has been approximately 30-40% male vs. 60-70% female. However, this ratio has changed from year to year with the addition of different numbers of male/female newcomers.

Pathological Studies

All monkeys, newborn to aged, which die or are terminated in our Center have complete autopsy and histological examination of major organs performed in the Center's Histopathology Division. An annual average of 80 autopsies of rhesus monkeys have been performed during the past 15 years. Histological studies have routinely been done by paraffin sectioning, H & E staining and thin plastic sectioning for light microscope. To study the incidence of age-related diseases, autopsy data from a total of 297 cases, 122 late adult (15 to 19 years old) and 175 aged monkeys (20 to 37 years old) were surveyed. For the study of cerebral amyloidosis, 81 brains of monkeys ranging in age from 16 to 37 years were used and immunocytochemistry of b-amyloid and amyloid precursor proteins was performed in the frontal, pre-frontal, temporal including hippocampal gyrus, and post- and pre-central gyri. Detailed methods of the amyloid staining were described in our previous paper (Uno *et al.*, 1996). For detection of iron deposits in the globus pallidus and substantia nigra, slices of formalin-fixed brains from a total of 32 monkeys, 15 brains from ages 6 to 19 years and 17 from ages over 20 years, were stained with the ferric ferrocyanide method (Berlin blue staining). The same staining was also applied to paraffin sections of the globus pallidus and substantia nigra regions.

Magnetic Resonance Image

A total of 18 monkeys, 11 of younger ages (2 to 12 years) and 7 aged monkeys (25 to 37 years) were examined by MRI of the brain for detection of multiple cerebral infarctions as well as iron deposits in the globus pallidus and substantia nigra. MRI is performed under anesthesia with a Ketamine/Xylazine mixture (7 mg/0.6mg per kg). The brain imaging was performed by the formulation of T-1 weighted, 60 serial coronal images, 1.3 mm thick, using a Signa scanner (1.5 tesla magnetic field), pulse sequence -3D SPGR with TR, repetition rate = 33 msec, and TE, echo time = 15 msec.

Some imaging was performed by T-2 dependent, TR = 2000 msec and TE = 90 msec with the same scanner.

Results

A. Survival Rate of Aged Rhesus Monkeys

For a longitudinal survey of the survival rate of our aged monkeys, a total of 85 individuals from 20 to 31 years of age in 1980 was selected and the annual populations of live monkeys and monkeys which had died during the year were counted. Figure 1 represents the annual shifting of the numbers of live and deceased monkeys for 15 consecutive years.

A total of 85 monkeys in 1980 was reduced to half the initial population after 5 years and to one fourth after 10 years. After 15 years, only 2 monkeys survived at 34 years of age. Our census records during the past 15 years revealed that the annual death rate averaged 15.6% (10 to 22%) in monkeys from 20 to 30 years and the rate increased to over 50% (23 to 90%) after 31 years of age. Two monkeys in our aging colony had attained the age of 37 years at the end of 1994; later these two monkeys died at 39 years. Clinically, the cause of death of these aged monkeys was either acute cardiac insufficiency or chronic deteriorated condition associated with marked weight loss, muscular atrophy, and loss of appetite and motility.

B. Age-Related Pathology in the Aging Macaque Colony

i. Incidence of age-related diseases

The incidence of various age-related pathological disorders was surveyed from autopsy records of the past 15 years that include a total of 175 aged macaques, 20 to 37 years, and 122 adult macaques, 15 to 19 years of age. Autopsy cases used in this survey were selected from spontaneous deaths or animals terminated due to severe incurable illness. The cases which were terminated for experimental purposes or were treated with experimental drugs, special diets, and non-diagnostic surgical procedures were eliminated from both the census and autopsy surveillance records.

The incidence of major age-related diseases in various organs is listed in Table 1 and Fig. 2. Compared to the adult group, all major geriatric diseases significantly increased in macaques over 20 years of age. The incidence further increased in higher age groups.

ii. Major geriatric pathology

a) Cardiovascular diseases

Although most species of nonhuman primates are highly susceptible to induction of atherosclerotic changes in the major arteries with high cholesterol diets, spontaneously developed atherosclerotic changes were not common in captive macaques fed commercial monkey chow (Purina Monkey Chow) which contains 5% fat (Wissler & Vesselinovitch

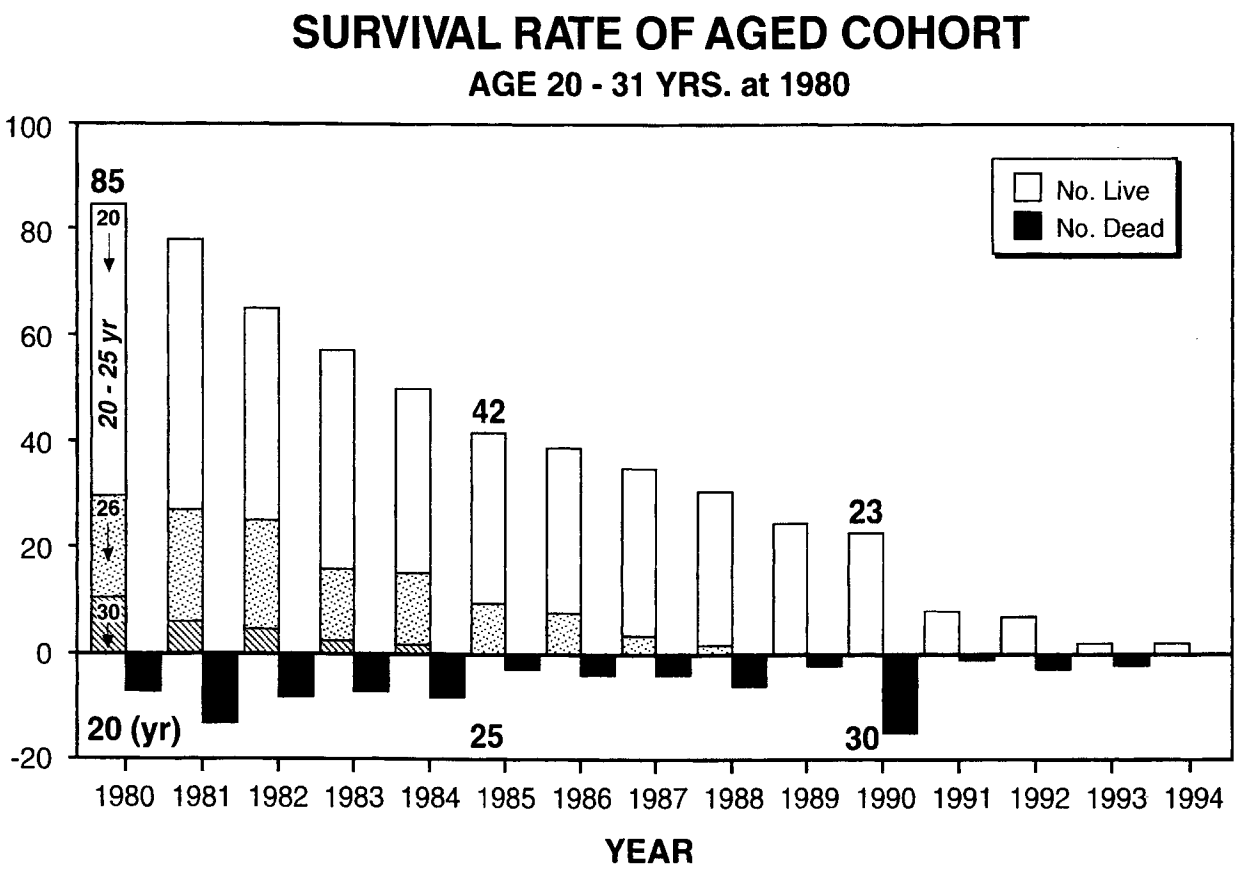

Figure 1. Annual survival and death records of an aged cohort, from 1980 to 1994. White bar: total number of live macaques, age from 20 to over 30, divided into sections, 20 to 25 (white), 26 to 29 (dotted) and over 30 years (shaded). The black bar below 0 line: total number of deaths.

Table 1: Incidences of major geriatric diseases.

Age Group (yr)	15-19	20-25	26-29	30-37
No. of autopsy	*122*	*109*	*37*	*29*
Coronary sclerosis	11 (9%)	39 (35.8%)	22 (59.5%)	25 (86.2%)
Myocardial fibrosis	8 (6.6%)	7 (6.4%)	6 (16.2%)	13 (44.8%)
Aorta, intimal thickening	7 (5.7%)	18 (16.2%)	8 (23.5%)	10 (34.5%)
Pulmonary emphysema	25 (20.5%)	46 (42.2%)	17 (45.9%)	20 (69.0%)
Degenerative joint disease	0.0%	11 (10.1%)	3 (8.11%)	8 (27.6%)
Osteoporosis	0.0%	12 (11.0%)	6 (16.2%)	12 (41.4%)
Cancer	4 (3.3%)	23 (21.1%)	6 (16.2%)	17 (58.6%)
Wasting disease syndrome	2 (1.6%)	12 (11.0%)	4 (10.8%)	4 (13.8%)
Atrophy of viscera	3 (2.5%)	9 (8.3%)	3 (8.1%)	7 (24.1%)
Obesity	25 (20.5%)	16 (14.7%)	4 (10.8%)	3 (10.3%)
Diabetes mellitus	1 (0.01%)	6 (5.5%)	1 (2.7%)	2 (6.9%)
Senile plaques (positive/total brains)	0/8 (0%)	5/24 (20.8%)	25/41 (60.9%)	8/8* (100%) *(over 33 years)
Cataracta*	*(0.5-19 yr)*	*(20-25 yr)*	*(26-28 yr)*	*(29-31 yr)*
(positive %/total cases)	0% (71)	58% (22)	77% (8)	100% (8)

*Kaufman and Bito, 1982

from Autopsy Records of Wisconsin Primate Res. Ctr., Pathology. 1980-1994

1973; Clarkson 1987). In our observation of aged macaques, the presence of intimal fibrous plaques constituted a major change in the aorta and major visceral arteries. Most of the intimal fibrous thickening with sporadic fatty streaks was found in the aortic arch and abdominal aorta. Severe ulcerative changes of the intima and deposition of cholesterol in the plaques were very rare, and if present, the changes were very slight. However, the incidence of these intimal fibrous plaques in the aorta and the major arteries gradually increased with age.

In our earlier study, the coronary arteries showed marked dilatation and a tortuous appearance in most major branches in approximately 60% of the

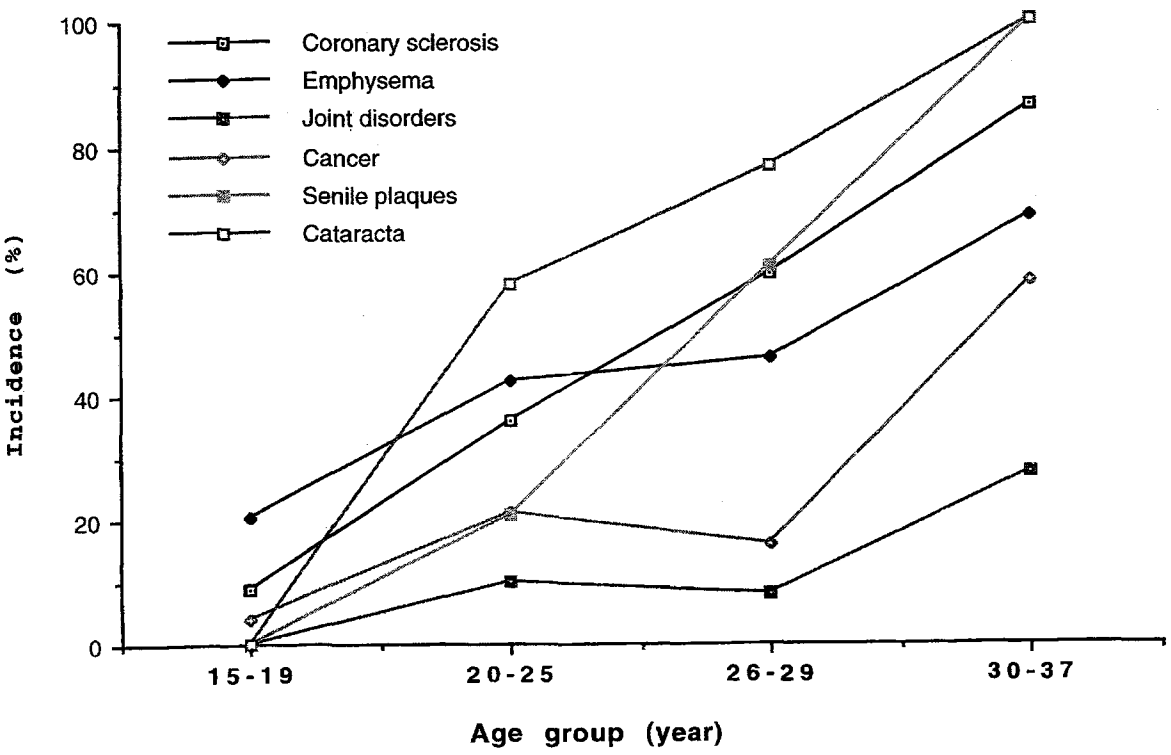

Figure 2. Incidence of major geriatric diseases, the rates of selected diseases from Table 1. The rates increase beyond 20 years of age.

macaques over 20 to 37 years of age (Uno and Poff 1983). These ectatic arterial branches involved no stenotic or aneurysmal bulging. Microscopically, the most conspicuous changes of the ectatic arteries were regressive changes of the media wall associated with a reduced number of smooth muscle cells and an increased number of interstitial fibrous cells. Intimal fibrous thickening was occasionally found above the regressed medial walls. Focal myocardial fibrosis showing distinct whitish scarry changes in the ventricular wall was found in approximately 30% of hearts involved with ectatic coronary arteries.

Despite very mild atherosclerotic change, interstitial fibrotic change accompanied by a reduced number of muscle cells in the medial wall of the coronary arteries appeared to be one of the prevalent types of aging outcome in captive macaques. Such coronary sclerosis occurred in more than 80% of the aged macaques over 30 years of age. Since the incidence of cardiovascular changes in human aged individuals varies greatly due to diverse environmental factors, heredity and ethnic groups, a comparison of the cardiovascular pathology of the human and macaque aging processes would not be relevant in establishing common biomarkers of chronological age and senescence.

b) Pulmonary emphysema

Subpleural bullae, multiple bubble-like expansions of the alveolar cavities, were seen mostly in the peripheral regions of the lobes in aged macaques. The incidence of emphysema significantly increased in macaques over 20 years of age (Table 1, Fig. 2). Since the lesions are mostly limited to the peripheral lobes, the changes may not cause life-threatening outcomes. In aged human beings, the complications of concurrent chronic bronchitis or bronchiectasia in emphysematous lungs are rather common and contribute to severe pulmonary disorder and death. In aged macaques, these complications are rarely encountered.

c) Musculoskeletal-skeletal disorders

A decrease in locomotor activity due to degenerative joint disorders such as spondylosis, kyphosis, and osteochondritis in major joints is a predictable age-related disease in aged macaques (DeRousseau *et al.* 1986). Such diseases in the musculoskeletal system are common in our aging colony of rhesus monkeys. Degenerative joint disease, such as osteoarthritis, spondylosis, and kyphosis, along with atrophy of skeletal muscle, caused a decrease in mobility beginning shortly after 20 years of age and

reaching approximately 30% incidence in older (postmenopausal to senescent) macaques (Table 1). These data included largely advanced cases, because the changes were determined by macroscopic observation at gross necropsy. In comparing the data to human skeletal changes, the conditions of housing and activity ranges in the captive macaques were quite different from those of human beings.

Osteoporosis is a rather common outcome in postmenopausal women (Davidson *et al.* 1982; Heaney 1983). In rhesus macaques, the incidence of osteoporosis in a free-ranging colony appeared to be higher than in female macaques living in indoor single or double occupancy cages (DeRousseau 1985). In our aged captive macaques, osteoporosis appeared to develop in approximately 11% of macaques of premenopausal ages; thereafter, the incidence gradually increased with further aging.

d) Cancer

The incidence of spontaneously developed cancer significantly increased in macaques over 20 years of age in our aging colony. A total of 47 cancers in various organs was found in 175 macaques of late adult and aged groups. In the adult population, only 4 cancers were encountered in 122 autopsied macaques from 13 to 19 years of age (Table 2). Among a total of 50 cancers, including 4 cases in adult macaques, 25 cancers developed in the colon. The breast (8) and uterine cervix (4) were other frequent sites in aged macaques. Sporadic occurrences of cancer were found in the pancreas, oral mucosa, skin, kidney, ovary, thyroid, and malignant lymphoma.

Table 2: Cancers in aged macaques.

Organ	*Cases*	*Site*	*Histopathology*
Digestive Tract	**30**		
Colon	25 (4)*	cecum, others	mucinous adenocarcinoma
Pancreas	3	head, body	adenocarcinoma
Oral mucosa	2	gingival wall	squamous cell carcinoma
Reproductive organs	**13**		
Breast	8	lateral quadrant	ductal carcinoma
Uterine cervix	4	cervix	squamous cell carcinoma
Ovary	1		mucinous cyst carcinoma
Others	**8**		
Skin	4	toe, penis, forearm	squamous cell ca., sweat gl. ca
Kidney	2	cortex	renal tubular cell carcinoma
Thyroid	1		papillary adenocarcinoma
Lymph node	1	mesenteric, ileocecal	histiocytic lymphoma
total	**51**		

175 autopsy cases (over 20 yr), * 4 cases in 13-19 yr

Although the lung and prostate were among the most frequent sites of cancer in the aged human population, cancers in these organs were not found in our aged macaques. Lung and prostate cancers in nonhuman primates were rarely reported in the literature (Giddens & Dillingham 1971; Engle & Stout 1940).

The incidence of, and death caused by, colorectal cancer in the United States are the third highest for cancer following prostate and lung cancer in men and lung and breast cancer in women. The incidence of colorectal cancer appears to significantly increase in the over 60 years-of-age population (Brody & Brock 1987; Finch 1990; Brock *et al.* 1990). In our aged macaques, the incidence of colon cancer markedly increased in macaques over 20 years of age compared to the adult age group (Table 3). Among aged macaques, the rate further increased with advancing age; 9.2% in animals 20 to 25 years, 13.5% in animals 26 to 29 years, and 20.7% in animals over 30 years of age. Among 25 cases of colon cancer in macaques, 18 cases occurred in females and the remaining 7 in males.

Most cancers in macaques were of a unicentric origin causing a napkin-ring constriction at the involved colonic region. Two cases of multicentric origin developed several fungiform growths associated with irregular thickening of the mucosa that involved chronic hyperplastic colitis. Two cases had metastasis to the mesenteric lymph nodes. The most common site of cancer was the cecum (12) and the other sites were the transverse-descending (9) and the recto-sigmoid (3) regions.

Compared to human colon cancer which develops most commonly in the rectosigmoidal region and also includes some cases associated with pre-existing benign polyps, the cancers in our macaques were largely found in the cecum and pre-cancerous polypoid lesions were not found.

A uniquely high incidence of spontaneously developed colon cancer is known in certain captive groups of the cotton-top tamarin. Recent extensive studies on the epidemiology and pathology of this disease described how the mortality rate from colon cancer progressively increased along with age in the tamarin (Clapp & Storer 1993). The cancer in tamarins at Oak Ridge Associated Universities (ORAU) develops in younger individuals and death caused by cancer rapidly increases after 4.5 years. Pathologically, most cancers in tamarins at ORAU develop from a unicentric origin with an occasional multicentric case.

The cotton-top tamarins in the New England Primate Center have also been known to have a rather high incidence of colon cancer which is associated mostly with chronic colitis (King *et al.* 1993). The rates of colon cancer in different age groups are very similar, 20.8 to 28.3% among infant (younger than 6 months), juvenile (6 months to 2 years), adult (2 to 5 years), and older adult (over 5 years of age) groups. The colony of cotton-top tamarins in the Psychology Department of the University of Wisconsin appears to have a very low incidence of colon cancer (Ziegler, personal communication). The diverse oncogenic backgrounds and environmental factors in captive colonies of cotton-top tamarins appear to be good models for the pathogenesis of human colon cancer. However, the age of onset in the cotton-top tamarin is generally younger than in human beings and macaques.

Table 3: Incidence in age groups and original sites.

Age group	*Case*	*Cancer/Total death (%)*	*Site*	*Location*
Adulthood				
13 yr	1		Transverse (TR)	Uni-centric
17 yr	2		Cecum (2)	Uni-, Multi-centric
19 yr	1		Cecum	Uni-
	4	4/126 (3.2%)		
Premenopausal				
20 yr	4		TR (2), Sigm, Rectum	Uni-
21 yr	1		Cecum	Uni-
23 yr	2		TR (2)	Uni-
24 yr	1		Cecum	Uni-
25 yr	2		Descend (2)	Uni-
	10	10/109 (9.2%)		
Menopausal, plus				
26 yr.	2		Cecum, TR	Uni-, Multi-
29 yr	3		Cecum (2), Sigm.	Uni-
	5	5/37 (13.5%)		
Senescence				
30 yr	4		Cecum (3), TR	Uni-(4), Metastasis (1)
36 yr	1		Cecum	Uni-, Metastasis
37 yr	1		Ascend.	Uni-
	6	6/29 (20.7%)		
Aging groups				
(20-37 yr)	21	21/175 (12%)	Original Sites	
			Cecum (12), Ascending (1)	
			Transverse (7), Descending (2)	
			Sigmoid (2), Rectum (1)	

The breast cancers which developed in our aged macaques were all of ductal origin (ductal carcinoma) and usually grew rather slowly as a single nodule or multiple nodules near the nipple. Two of seven cases resulted in metastasis to the lung and the remaining four cases grew within the breast tissues.

Four cases of uterine cancer developed in the cervix and two of them resulted in either massive growth into the abdominal cavity or metastasis into the lung. The remaining two cases were found within the cervical tissues at an early invasive stage. Histologically, these cancers consisted of squamous carcinoma. Although the uterine cancer of postmenopausal women often develops from the endometrium of the uterine body, the cancers in our aged macaques all developed from the cervical mucosal tissues.

The cancers which developed in the skin and oral mucosa consisted of squamous carcinoma. Nodular growths of cancers developed either in the toe, penis, or the gingiva invading the mandibular bone. Postsurgical recurrence near the original site was encountered in two cases of skin cancer.

Overviews of spontaneous tumors in nonhuman primates have been published by many investigators (McClure 1980). A recent literature survey for spontaneous tumors includes both benign and malignant tumors in various organs in a wide group of species, from tupia to apes (Beniashvili 1989). However, the author mentioned that the age-specific incidence of neoplasms in nonhuman primates is impossible to ascertain from the literature.

Wasting disease syndrome manifesting loss of appetite, progressive weight loss, atrophy of skeletal muscles and adipose tissues, and low vital activities was a rather common terminal condition in aged macaques (Weindruch *et al.* 1995). A majority of aged monkeys in the terminal stage manifested this syndrome associated with the above described major pathological diseases in various organs. Obesity and diabetes mellitus were not major outcomes in aged macaques.

C. Pathology of the brain in aged macaque

i) Multiple cerebral infarction

In examination by magnetic resonance imaging, multiple cerebral infarctions were detected in 7 aged macaques (25 to 37 years). Multiple infarctions in MRI examinations and equivalent pathological lesions (cerebral softening) in a 30-year-old male monkey were studied in the postmortem brain (Fig. 3). The infarctions were commonly found in the parietal and temporal lobes and occasionally in the cerebellar cortices and thalamic nucleus. MRI examination of 11 juvenile and young adult macaques (2 to 13 years of age) showed no such lesions (Table IV). Clinically, 2 of the above 7 aged macaques had suffered from paralysis of the limbs. The postmortem brains of 3 aged macaques manifested multiple infarctions in the regions corresponding to the MRI (Uno *et al.* 1990). The hearts of these 3 macaques had single or multiple foci of myocardial fibrosis accompanied by coronary sclerosis. Although cere-

brovascular changes in aged macaques appear to be very rare, relative hypoxia in certain brain regions may cause such multiple infarctions.

Table 4: Cerebral infarction and Pallidonigral iron deposit.

Age/sex	*MRI Abnormalities*	*Images/date*	*Remark*
2 yr/m (5)*	no abnormality	T1 Oct. 92	live
2 yr/f (3)	"	T1 Oct. 92	"
8 yr/m	"	T1 Dec. 93	"
10 yr/m	"	T1 Dec 93	"
12 yr/m	"	T1 Dec. 93	"
25 yr/m	**MI; P-O lobes GP-SN (darkening)**	**T1 Aug. 93**	**live**
28 yr/m	**MI; O lobe GP-SN**	**T1, T2. Oct. 92**	**live**
30 yr/m	**MI; P-O lobes GP-SN**	**T1, T2. Aug. 93**	**pm***
30 yr/m	**MI; O lobe GP-SN**	**T1 Sep. 93**	**live**
30 yr/m	**MI; P-T lobes, cerebell, thalamus. GP-SN**	**T1, T2. Mar. 89**	**pm**
30 yr/f	**MI; O lobe GP-SN**	**T1 Dec. 92**	**pm**
37 yr/m	**MI; P-O lobes, hydroceph, GP-SN**	**T1, Dec. 93**	**pm**

* (case)

MI; multiple infarction
GP-NS; iron deposit in Globus pallidus and Substantia nigra
P; parietal, O; occipital, T; temporal lobes
T1; T1 weighted, Spin density *pm; with postmortem study
T2; T2 dependent, 2nd Echo

ii) Pallidonigral deposit of iron pigment

MRI examination of the aged macaque brains showed a reduced signal, a distinct darkening, of the globus pallidus and substantia nigra in the above 7 macaques examined for cerebral infarction (Fig. 4). Pathological changes in the areas of these iron deposits in the globus pallidus and substantia nigra were well documented in macaque brains (Bronson & Schoene 1980; Gliatto & Bronson 1993; Cork & Walker (1993). Aspects of these lesions in human and macaque brains were also compared (Schoene *et al.* 1977).

MRI examination of human brains has revealed similar darkening in both the globus pallidus and substantia nigra in patients diagnosed with Hallervorden-Spatz disease (Angelini *et al.* 1992; Trussart *et al.* 1993). The disease causes varying degrees of mental and physical deterioration, and abnormal iron deposition has been demonstrated in the globus pallidus, substantia nigra, and red nucleus (Porter-Grenn *et al.* 1993). However, the disease occurs in a wide age range in human patients.

Slices of formalin-fixed brains of a total of 32 rhesus macaques, 15 brains of animals from 6 to 19 years, and 17 of animals over 20 years of age, were stained with the ferric ferrocyanide method (Berlin blue staining) for iron deposits. Positive staining for

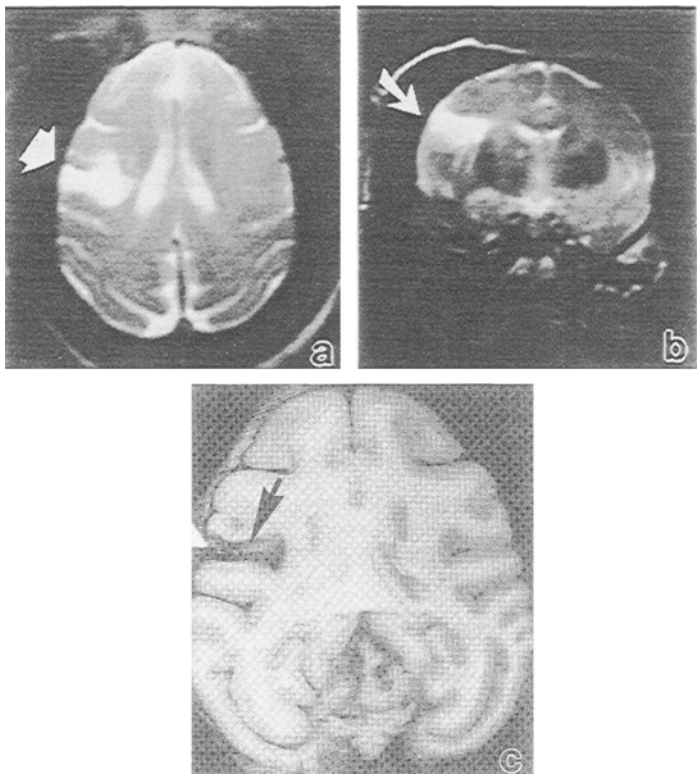

Figure 3. Magnetic resonance images (T-2 dependent, Tr, repetition rate=2000 msec, and TE, echo time=90msec, using a GE scanner, 1.5 tesla). Opaque shadows in the left lateral temporal lobe in horizontal (a, arrow) and coronal (b, arrow) planes. An area of cerebral softening in the temporal lobe of horizontally sliced brain (c, arrow) corresponds with MRI images.

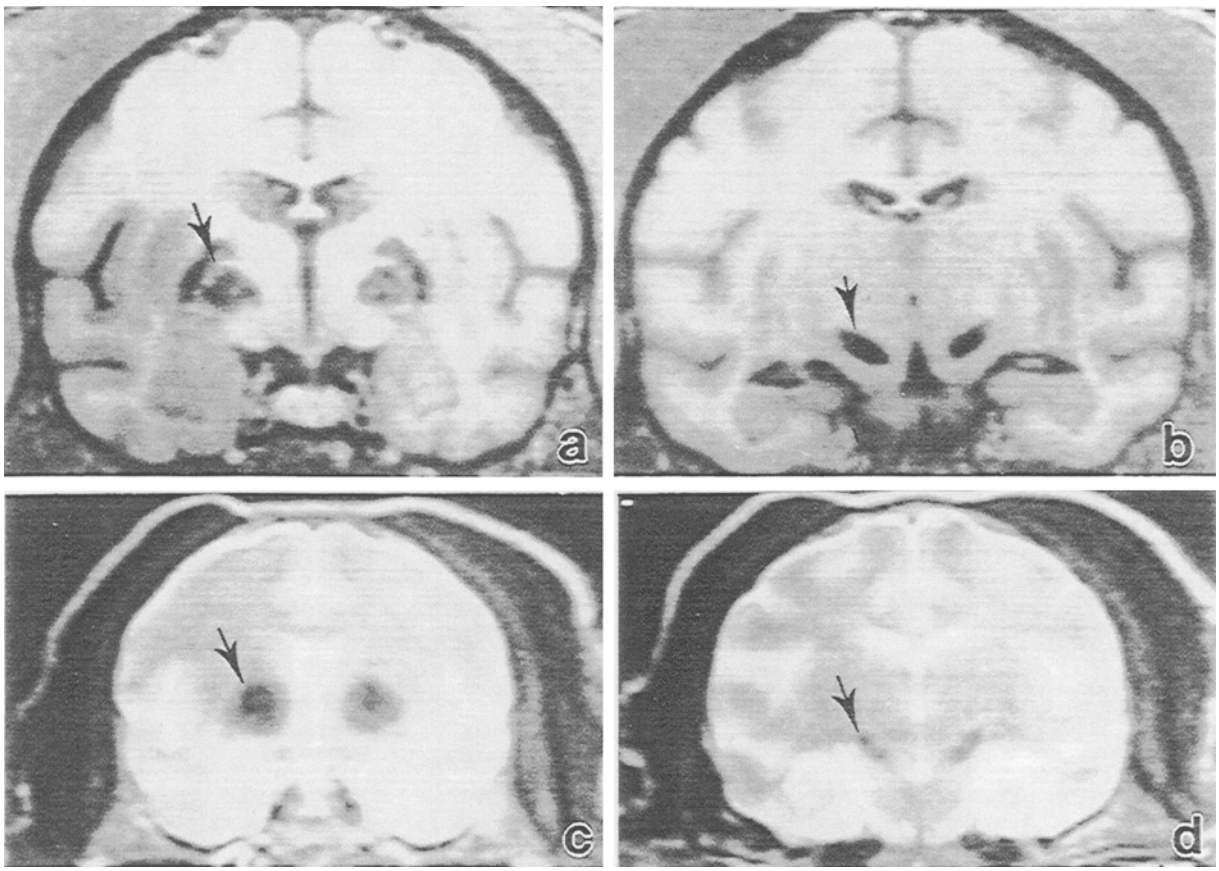

Figure 4. Magnetic resonance images of four coronal planes, [T-1, Tr=2000 msec, TE=20 msec. (a) and (b)] and T-2 dependent (c) and (d) showed reduced signal (black shadows, arrows) in the globus pallidus (a and c) and substantia nigra (b and d).

iron pigments in the globus pallidus and substantia nigra corresponded to brownish discolored areas in both regions. Of 16 brains in the adult group, 7 cases were positive for iron pigments in both regions; the youngest age of appearance was 12 years, and monkeys 17 to 19 years of age were all positive. With the exception of one case at 20 years of age, the remaining 26 brains from 20 to 32 years of age were all positive. Microscopically, positive iron staining was found in hemosiderin-laden macrophages in the perivascular spaces and in scattered microglia cells in the globus pallidus and substantia nigra. Depletion or degeneration of the neurons, softening, and fresh hemorrhagic foci were not found in these brain regions (Uno *et al.* 1994).

These peculiar lesions detected by both MRI and pathological examination in the pallidonigral nuclei in macaque brains appeared to be age-related, but the age at onset was much younger than that for other geriatric diseases.

iii) Beta-amyloid (senile) plaques in the aged macaque brain

Our studies in a total of 81 brains of late adult and aged macaques, 16 to 39 years of age, revealed that plaques appeared in the brains of monkeys over 20 years of age. Brains from eight adults, 16 to 19 years of age, had no detectable plaques. In the aged group, the incidence was 20.8% in 24 brains of monkeys of premenopausal age (20 to 25 years), 60.9% in 41 brains of postmenopausal age (26 to 31 years), and 100% in 8 brains of senescent age (33 to 37 years of age) (Table V). Of a total of 38 plaque-positive brains in aged macaques, 10 were associated with cerebral beta-amyloid angiopathy (Uno *et al.* 1996). The density of plaques in the cortical regions generally increased with advancing age. Unlike some aged macaques reported in the literature which showed plaques densely distributed over the entire cerebral cortical region, in the brains of our aged monkeys the plaques were most frequently found in the basal prefrontal (supraorbital) gyri and amygdala and occasionally in the parietal and hippocampal gyri (Table 6).

Although we have no definitive data on the cognitive behavior of our aged macaques, anatomically the brains of these aged monkeys showed no decline of weight and no visible atrophy of the cerebral cortices. Histologically, the cortical neurons showed no degenerative changes and their zonal arrange-

ments were well-organized, even in the cortical regions containing many plaques. MRI examination of 7 aged macaque brains also showed no atrophic changes in the cortical gray matter and basal ganglia, and no dilatation of the ventricles, with the exception of the areas showing cerebral infarction. The severe syndrome of dementia in human Alzheimer's and senile dementia patients is likely caused by atrophy of the cerebral cortices accompanied by neurofibrillar degeneration of cortical neurons and axodendritic branches, and characterized by densely distributed amyloid plaques.

Development of amyloid plaques in the cerebral cortex and amyloid angiopathy in the brain and meningeal vessels has been known as a rather common outcome in aged Old World monkeys as well as in a South American monkey, the squirrel monkey (Wisniewski *et al.* 1973; Price *et al.* 1985; Struble *et al.* 1985; Iwata 1986; Cork *et al.*, 1990; Walker *et al.*, 1990; Uno & Walter, 1993; Uno *et al.* 1996). The appearance of beta-amyloid plaques and neurofibrillary tangles of cerebral cortical neurons are major pathological changes in the brains of aged human beings suffering from Alzheimer's disease as well as senile dementia of the Alzheimer type. Although most investigators agree that aged monkey brains have no detectable neurofibrillary tangles, development of amyloid plaques is a common age-related pathology in aged brains. Since the amyloid proteins in the plaques of monkeys show immunocytochemical cross-reactivity with human beta-amyloid as well as amyloid precursor protein, the lesions in monkeys are considered to be a good model for studies on the pathogenesis of cerebral beta-amyloidosis in the aging human brain (Uno *et al.*, 1996). The aged monkeys living in a free-ranging colony appeared to have a more dense and wider distribution of the plaques than our aged macaques kept in single cages for 2 to 3 decades (Iwata 1986).

Table 5: Incidence of cerebral amyloidosis.

Age Group	*Total Case*	*Cerebral Lesions*	
		Plaques	*Angiopathy*
16-19 yr	**8**	0	0
20-25 yr	**24**	**5** (20.8%)	**2** (8.3%)
26-31 yr	**41**	**25** (60.9%)	**5** (12.2%)
33-37 yr	**8**	**8** (100%)	**3** (37.5%)
Total	**81**	**38** (46.9%) **over 20 yr** (52.0%)	**10** (12.3%) (13.6%)

Table 6

Brain region (Brodmann area*)	*Plaque positive brain* (%/total positive brain =38)
Amygdala (+ 21, 28 areas)	**27 (71.1%)**
Basal prefrontal (10, 11, 25)	**26 (68.4%)**
Hippocampus (+ 27 area)	**12 (31.5%)**
Pre-, Post-Central (4, 3, 1)	**4 (10.5%)**

*Markowitsch, 1988

DISCUSSION

Comparative Biosenescence and Longevity

Prolonged longevity in modern human society is largely due to advanced medical practices and various preventive measures for age-related diseases in well-developed countries. However, the rates of age-adjusted deaths from colorectal, prostate, breast, ovarian, pancreatic, and liver cancer have not significantly changed in the United States since 1930, with the exception of a continuously increased death rate due to lung cancer from 1940 to 1990 in men and from 1970 to 1990 in women, and continuously decreasing rates of stomach and uterine cancers from 1930 to 1990 (Cancer Facts & Figures, 1996). Most cancers which develop in the major visceral organs are age-related outcomes in the human population over 65 years of age and major geriatric diseases such as cancers, cerebrovascular diseases, pneumonia, arteriosclerosis, diabetes mellitus, and nephritis, significantly increased in this age group (Brody *et al.* 1987; Brock *et al.* 1990).

Based on our pathological data in aged captive rhesus monkeys, the incidence of various geriatric disorders rapidly increases in macaques over 20 years of age. According to a longitudinal survey of a cohort in our aged macaque colony, half of the population at 20 years of age died by 25 years, and the other half died by 30 years of age. In our aging colony, although a few monkeys survived over 33 years and the greatest longevity was 39 years (2 female macaques), an increased incidence of major geriatric disorders in macaques after 20 years of age caused increased deaths and a stepwise decrease of the surviving population (Fig. 1). Our longevity record in rhesus monkeys was similar to that previously reported in this species (Cutler, 1976; Cutler, 1978; Bowden & Jones, 1979; Finch, 1990). Two rhesus monkeys in our aging colony recently died at 39 years of age.

Clinical Signs in Aged Rhesus Monkeys

As in human aging, the external appearance of aged macaques manifests a great degree of individual variation and is usually an unreliable predictor of chronological age. Among typical aging changes, dried and wrinkled facial skin associated with telangiectasia, tooth decay, atrophy of the skeletal muscles, and varying degrees of kyphosis were signs of aging in captive monkeys over 25 years of age. Mean body weight showed a gradual decline after 24 years in male and 30 years in female macaques. Idiopathic wasting syndrome associated with a loss of body weight and generalized atrophy of skeletal muscles and adipose tissues significantly increased in macaques over 20 years of age (Weindruch *et al.* 1995).

Ophthalmological examination of a total of 109 rhesus monkeys ranging in age from 6 months to 31 years in our Center revealed that detectable degrees of cataracts were found in all examined animals (38 cases) over 20 years of age and advanced cataracts appeared in animals over 26 (15%) and over 29 (38%) years of age (Kaufman & Bito 1982). Kaufman's group also studied presbyopia, the age-dependent decline in visual accommodative amplitude, in the above groups of rhesus monkeys. The age-dependent loss in accommodative amplitude exhibited progressive patterns in macaque aging similar to those in human aging; typical declines occurred in macaques after 25 years and in human beings after 40 to 45 years of age (Kaufman & Bito 1983).

These studies, using a relatively large number of monkeys in a wide age range and clearly showing geriatric outcomes in the lens and visual functions, are reliable markers of age-related changes and illustrate the difference between chronological age and the bioaging process of macaques. The data also provide useful parameters for comparing relative age and biosenescent differences between human beings and rhesus monkeys.

Menopausal Syndrome in Aged Macaques

The occurrence of menopause appears to be the most universal phenomenon with the least age variation in both women and female macaques. The event involves a decreased number of annual menstrual cycles and eventual cessation of the menses, a declining of conceptual capability, a change of gonadotropin levels, and a declining or cessation of ovarian folliculogenesis. With the exception of the presence of conditions such as endometriosis, endometrial polyps, and uterine and ovarian disorders, the number of annual menstrual cycles begins to decrease after the age of 22 to 25 years in macaques (Hodgen *et al.* 1977; Eisele, personal communication). In examination of 4 macaques over 24 years of age, cessation of menstruation occurred in 2 by 24 years, and in the remaining 2 by 27 years of age (Walker 1995). The highest age for maintenance of the menstrual cycles and conception was recorded in 26-year-old rhesus monkeys in the Wisconsin Primate Center (Uno and Walker 1993; Eisele, personal communication).

Sustained high levels of serum luteinizing hormone (LH) are known as a marker of menopause and ovarian involution in both women and female rhesus monkeys. Recent studies in both indoor and outdoor free-ranging captive rhesus as well as Japanese monkeys revealed that levels of LH were slightly elevated after 21 years and further elevation to 5 to 6 times adult levels occurred after 26 years in both species (Walker 1995; Nozaki *et al.* 1995). However, these studies indicated that the longitudinal levels of LH in most postmenopausal macaques occasionally dropped to a low range. Thus, a few times measurements of LH level in aged macaques may not be sufficient to determine the status of the ovarian-pituitary axis. Both studies also revealed that levels of estrogen declined in these postmenopausal monkeys after 26 years of age.

In Caucasian women, the median age of menopause has been established at between 49 and 51 years and the levels of serum LH as well as FSH significantly rise after menopause (Timiras & Sentenac 1995). However, it has also been documented that these high levels of gonadotropins begin to decline after the age of 60 (LH) and 70 to 80 years (FSH). LH levels in our monkeys became elevated after 26 years of age and the high levels of LH were sustained in 31- and 36-year-old monkeys (unpublished data). The elevation of gonadotropin in the peri- and postmenopausal period is essentially dependent on the declining and eventual cessation of ovarian folliculogenesis which results in a reduced production of estrogen and progesterone from the follicular cells. During the perimenopausal period the levels of gonadotropin appear to increase prior to significant declines in estrogen levels, suggesting the possibility of a reduction in pituitary response to negative feedback regulation by secretory products of the ovary as part of the aging process (Winston *et al.* 1987).

Although advances in modern techniques such as fiber-optic endoscopy and ultrasonography are available for observation of longitudinal changes in ovarian folliculogenesis, limited data is available for adult monkeys (Morgan *et al.* 1987). For the study of ovarian folliculogenesis, histological observation is still the most reliable means to determine the status of ovarian involution. Van Wagenen and Simpson first described the involuted ovaries of a 26.5-year-old rhesus monkey; the ovary was extensively scarred and contained no mature follicles or corpora lutea (Van Wagenen & Simpson, 1965). Our morphometric studies of ovarian folliculogenesis in adult (16-18), perimenopausal (25), and postmenopausal (30-31 year old) monkeys revealed that proportional numbers of primary, secondary, and tertiary follicles were significantly reduced in perimenopausal ovaries compared to those in adults. However, the ovaries in the perimenopausal macaques showed the presence of matured Graafian follicles and corpora luteum. In postmenopausal monkeys the ovaries contained extremely reduced numbers of primary follicles showing no signs of maturation in dense interstitial spindle cells. Such scarred ovarian tissues remained only in the outer zone, and the expanded hilum region consisted largely of intermingled sclerotic arteries and veins (Collins *et al.* 1983).

Thus, in rhesus and Japanese macaques, it is now generally agreed that by the age of 20 years reproductive activities become reduced and menopause occurs at 26 to 27 years (Walker 1995; Nozaki *et al.* 1995). The menopausal period of women is known to be 49 to 51 years of age. Menopause, the cessation of reproductive activity, represents the least age variation among other biomarkers of age-related outcomes in women as well

as female macaques. Thus, the age of menopause has often been used to define the chronological age corresponding to biosenescence in different mammalian species. However, the age of menopause in women generally does not represent a turning point from active adulthood to a geriatric lifestyle. In the current human aged population most geriatric disorders or senile phenomena begin to appear by 65 years of age, about 15 years after menopause. In contrast, our studies in aged macaques have revealed that major geriatric pathology starts to develop by 20 years of age which is approximately 5 years before menopause. After the postmenopausal period, despite living in captivity with good care, many monkeys die due to spontaneously developed pathological conditions and their complications. The average life expectancy of our captive macaques appears to be several years after their menopause.

COMMENT

Despite 15 years' cumulative data on geriatric pathology in 175 aged macaques, the numbers representing morbidity rates for various geriatric diseases were far lower than those surveyed in human standard studies. Additionally, there are differences between our captive rhesus monkeys and the human aged population in evolutional predispositions as well as environmental factors which contribute to major age-related diseases. For example, the incidence of lung and prostatic cancers in the human aging population is extremely high, but those cancers are very rare in aged macaques. Aging macaque brains develop beta-amyloid plaques and angiopathy similar to those seen in human Alzheimer's disease or senile dementia patients, but neurofibrillary tangles as well as atrophy of cerebral cortices and ganglia are not detected in aged macaque brains. Although diverse ethnic, environmental, and predisposing factors contribute to cause these aged-related pathologies in the human population, our aged rhesus monkeys mostly have been born in our facility and have lived in indoor cages with a controlled diet and no exposure to the outside environment. Thus, with minimal exposure to various environmental factors, the diseases which develop in our aged macaque colony can be considered to be natural outcomes of aging.

For studies of various preventive measures for aging or age-related diseases in human society, these data on aged macaques will constitute a baseline or background on major geriatric diseases for natural aging outcomes versus the events caused by various exposures to environmental risk factors. Furthermore, these biosenescence markers in aged macaques can be useful for future paradigms in many fields of comparative biomedical studies of aging as well as for estimations of age in both feral and captive aged macaques.

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REFERENCES

Angelini, L., Nardocci, N., Rumi, V., Zorzi, C., Strada, L., and Savoiardo, M.: Hallervorden-Spatz disease: Clinical and MRI study of 11 cases diagnosed in life. J. Neurol., 239: 417-425, 1992.

Beniashvili, DS.: An overview of the world literature on spontaneous tumors in nonhuman primates. J. Med. Primatol., 18: 423-437, 1989.

Bowden, D.M.,, and Jones, M.L.: Aging research in nonhuman primates, in Aging in Nonhuman Primates, edited by Bowden, D.M., New York, Van Nostrand Reinhold Co., 1979, pp. 1-13.

Brock, D.B., Guralnik, J.M., and Brody, J.A.: Demography and epidemiology of aging in the United States, in Handbook of the Biology of Aging, edited by Schneider, E.L., and Rowe, J.W., third edition, San Diego, Academic Press, Inc., 1990, pp. 3-23.

Brody, J.A., Brock, D.B., and Williams, T.F.: Trends in the health of the elderly population, in Annual Review of Public Health, Vol. 8, 1987, pp. 211-234.

Bronson, R.T., and Schoene, E.C.: Spontaneous pallidonigral accumulation of iron pigment and spheroid-like structures in macaque monkeys. J. Neuropathol. Exp. Neurol., 39: 181-196, 1980.

Cancer Facts & Figures – 1996, American Cancer Society, Inc., Atlanta, GA.

Clapp, N.K., and Storer, J.B.: Death rates with age from all causes and from colonic carcinoma in wild-caught and colony-born cotton-top tamarins. Chapter 15 in A Primate Model for the Study of Colitis and Colonic Carcinoma, edited by Clapp, N.K., CRC Press, Boca Raton, 1993, pp. 221-230.

Clarkson, T.B., Weingand, K.W., Kaplan, J.R., and Adams, M.R.: Mechanisms of atherogenesis. Circulation, 76 (Suppl. I): I20-I28, 1987.

Collins, K., Uno, H., and Dierschke, D.J.: Ovarian follicle populations in peri- and post-menopausal rhesus monkeys. Fed. Proc., 42: 316, 1983.

Cork, L.C., Masters, C., Beyreuther, K., and Price, D.L.: Development of senile plaques. Relationships of neuronal abnormalities and amyloid deposits. Am. J. Pathol., 137: 1383-1392, 1990.

Cork, L.C., and Walker, L.C.: Age-related lesions, nervous system. In Nonhuman PrimatesII, edited by Jones, T.C., Mohr, U., and Hunt, R.D., New York, Springer-Verlag, pp. 173-183.

Cutler, R.G.: Evolution of longevity in primates. J. Human Evolution, 5: 169-202, 1976.

Cutler, R.G.: Evolutionary biology of senescence, in The Biology of Aging, edited by Behnke, J.A., Finch, C.E., and Moment, G.B., New York, Plenum Press, 1978, pp. 311-360.

Davidson, J., Ross, R.K., Paganini-Hill, A., Hammond, C.D., Siiteri, P.K., and Judd, H.L.: Total and free estrogen and androgens in postmenopausal women with hip fractures. J. Clin. Endocrinol. Metab., 54: 115-120, 1982.

DeRousseau, C.J.: Aging in the musculoskeletal system of rhesus monkeys: II. Degenerative joint disease. Amer. J. Phys. Anthrop., 67: 1771-1784, 1985.

DeRousseau, C.J., Bito, L.Z., and Kaufman, P.L.: Age-dependent impairments of the rhesus monkey visual and musculoskeletal systems and apparent biohavioral consequences, in The Cayo Santiago Macaques: History, Behavior and Biology, edited by Rawlins, R.G., and Kessler, M.J., Albany, SUNY Press, 1986, pp. 233-251.

Engle, E.T., and Stout, A.P.: Spontaneous primary carcinoma of the prostate in a monkey (Macaca mulatta). Am. J. Cancer, 39: 334-337, 1940.

Finch, C.E.: Longevity, senescence, and the genome. Chicago, The University of Chicago Press, 1990, p. 163.

Giddens, E.W., and Dillingham, L.A.: Primary tumors of the lung in nonhuman primates. Veterinary Pathol., 8: 467-471, 1971.

Gliatto, J.M.,, and Bronson, R.T.: Spontaneous pallidonigral spheroids and iron pigment accumulation, macaques, in Nonhuman Primates II, edited by Jones, T.C., Mohr, U., and Hunt, R.D., New York, Springer-Verlag, 1993, pp. 183-187.

Heaney, R.P., Recker, R.R., and Saville, P.D.: Menopausal changes in calcium balance performance. Nutrition Rev., 41: 86-89, 1983.

Hodgen, G.D., Goodman, A.L., O'Connor, A., and Johnson, D.K.: Menopause in rhesus monkeys: Model for study of disorders in the human climacteric. Am. J. Obstet. Gynecol., 127: 581-584, 1977.

Iwata, T.: Studies on senile changes in the brains in monkeys (Macaca fuscata). I. On senile plaque. Acta Sch. Med. Univ. Gifu, 34: 540-550, 1986.

Kaufman, P.L., and Bito, L.Z.: The occurrence of senile cataracts, ocular hypertension and glaucoma in rhesus monkeys. Exp. Eye Res., 34: 287-291, 1982.

Kaufman, P.L., Bito, L.Z., and DeRousseau, C.J.: The development of presbyopia in primates. Trans. Ophthal. Soc. U.K., 102: 323-326, 1983.

King, N.W., Johnson, L.D., and Sehgal, P.K.: The prevalence of idiopathic colitis in the New England Regional Primate Research Center cotton-top tamarin (Saguinus Oedipus) colony, Chapter 5 in A Primate Model for the Study of Colitis and Colonic Carcinoma, edited by Clapp, N.K., CRC Press, Boca Raton, 1993, pp. 101-112.

Lapin, B.A.: Use of nonhuman primates in cancer research. J. Med. Primatol., 11: 327-341, 1982.

Lapin, B.A., Krilova, R.I., Cherkovich, G.M., and Asanov, N.S.: Observations from Sukhumi, Chapter 2 in Aging in Nonhuman Primates, edited by Bowden, D.M., New York, Van Nostrand Reinhold Co., 1979, pp. 14-37.

McClure, H.M.: Neoplastic diseases in nonhuman primates: Literature review and observations in an autopsy series of 2,176 animals, in The Comparative Pathology of Zoo Animals, edited by Montal, R.J., and Migaki, G., Washington, D.C., Smithsonian Institution Press. 1980, pp. 549-565.

Morgan, P.M., Hutz, R.J., Kraus, E.M., Cormie, J.A., Dierschke, D.J., and Bavister, B.D.: Evaluation of ultrasonography for monitoring follicular growth in rhesus monkeys. Theriogenol., 27: 769-779, 1987.

Nozaki, M., Mitsunaga, F., and Shimizu, K.: Reproductive senescence in female Japanese monkeys (Macaca fuscata): Age- and season-related changes in hypothalamic-pituitary-ovarian functions and fecundity rates. Biol. Reprod., 52: 1250-1257, 1995.

Porter-Grenn, L., Silbergleit, R., and Mehta, B.A.: Hallervorden-Spatz disease with bilateral involvement of globus pallidus and substantia nigra: MR demonstration. J. Computer Assisted Tomography, 17: 961-963, 1993.

Price, D.L., Cork, L.C., Struble, R.G., Kitt, C.A., Price, D.L. Jr., Lehmann, J., and Hedreen, J.C.: Neuropathological, neurochemical and behavioral studies of the aging nonhuman primate. Chapter 7 in Behavior and Pathology of Aging in Rhesus Monkeys, New York, Alan R. Liss, 1985, pp. 113-135.

Schoene, W.C., Bronson, R.T., Dooling, E.C., Richardson, E.P.: Spontaneous pallido-nigral spheroid pigmentary degeneration in monkeys: An analog of human Hallervorden-spatz disease. J. Neuropathol. Exp. Neurol., 36: 628, 1977.

Struble, R.G., Price, D.L. Jr., Cork, L.C., and Price, D.L.: Senile plaques in cortex of aged normal monkeys. Brain Res., 361: 267-275, 1985.

Timiras, P.S., and Sentenac, J.: Aging of the female reproductive system, Chapter 7 in Hormones and Aging, edited by Timiras, P.S., Quay, W.D., and Vernadakis, A., Boca Raton, CRC Press, 1995, pp. 121-134.

Trussart, V., Leboucq, N., Carlander, B., Billiard, M., and Castan, P.: Hallervorden-Spatz syndrome and MRI: The "tiger's eye." One Case. J. Neuroradiol., 20: 70-75, 1993.

Uno, H., Alsum, P.B., Dong, S., Richardson, R., Zimbric, M., Thieme, C.S., and Houser, W.D.: Cerebral amyloid angiopathy and plaques and visceral amyloidosis of aged macaques. Neurobiol. Aging, 17: 275-281, 1996.

Uno, H., Houser, W.D., and Holden, J.E.: Magnetic resonance images of the cerebral infarction of an aged rhesus macaque: Comparison between T1 and T2 images and neuropathology. Neurosci. Abst., 16(2) 1160, 1990.

Uno, H., Kao, Y., Baker, E., Shelton, S., and Holden, J.: MRI study of multiple infarctions and iron deposits in the globus pallidus in aged rhesus monkeys. Neurosci. Abst., 20-I: 180, 1994.

Uno, H., and Poff, B.: Coronary arterial ectasia, a predominant type of coronary sclerosis in aged captive rhesus monkeys (Macaca mulatta). Am. J. Path., 111: 315-322, 1983.

Uno, H., and Walker, L.C.: The age of biosenescence and the incidence of cerebral b-amyloidosis in aged captive rhesus monkeys. Ann. N.Y. Acad. Sci., 695: 232-235, 1993.

Van Wagenen, G., and Simpson, M.E.: Embryology of the ovary and testis. Homo sapiens and Macaca mulatta. New Haven, Yale Univ. Press, 1965.

Walker, L.C., Masters, C., Beyreuther, K., and Price, D.L.: Amyloid in the brains of aged squirrel monkeys. Acta Neuropathol., 80: 381-387, 1990.

Walker, M.L.: Menopause in female rhesus monkeys. Am. J. Primatol., 35: 59-71, 1995.

Weindruch, R., Kemnitz, J.W., and Uno, H.: Interspecies variations in physiologic and antipathologic outcomes of dietary restriction, in Dietary Restriction: Implications for the Design and Interpretation of Toxicity and Carcinogenicity Studies, edited by Hart, R.W., Neumann, D.A., and Robertson, R.T., Washington, D.C., ILSI Press, 1995, pp. 351-364.

Winston, L.A., Dierschke, D.J., and Robinson, J.A.: Gonadotropins rise before estrogen declines in rhesus monkeys prior to menopause. Endocrine Soc. Absts., 1987, No. 873.

Wisniewski, H.M., Ghetti, B., and Terry, R.D.: Neuritic (senile) plaques and filamentous changes in aged rhesus monkeys. JNEN, 32: 566-584, 1973.

Wissler, R.W., and Vesselinovitch, D.: Differences between human and animal atherosclerosis, in Atherosclerosis III, edited by Schettler, G., and Weigel, A., New York, Springer, 1973, pp. 319-325.