have been previously reported by one of us (4).

Summary. Our findings support the principle that radiation produces its major effect on hemopoiesis and not on the circulating RBC. The changes found in the $DF^{32}P$ curves can be explained on the basis of bone marrow depression after irradiation.

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Isolation and Characterization of *Mycoplasma arginini*: spec. nov.* (33351)

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Ten strains of *Mycoplasma* were isolated from brain tissues of a scrapie infected sheep and mouse, joint exudate of an arthritic goat, and cell cultures established from human, chimpanzee, and dog tissues. These strains were related to each other, but were unrelated to known species of *Mycoplasma*. The present report characterizes the new strains, proposes that they be classified as *Mycoplasma arginini* and discusses their ecological significance.

Materials and Methods. Media for isolation. Hayflick (1), BYE (2), and BBL (3) broth and agar media were used under aerobic and anaerobic (5% CO_2 in nitrogen) conditions (2). See Table I for media used for isolation of individual strains.

Media for metabolic reactions. Arginine utilization reaction: Hayflick and BYE broth media were supplemented with 10 m*M* arginine, 10 m*M* glutamine, vitamins (4), 0.002% phenol red, and adjusted to pH 7.1.

Glucose fermentation reaction: Hayflick broth medium was supplemented with 0.5% glucose, 10 m*M* glutamine, vitamins, 0.004% cresol red, and adjusted to pH 7.5.

Urea metabolic reaction: Hayflick broth medium was supplemented with 0.5% urea, 0.002% phenol red, and adjusted to pH 6.0. Utilization of arginine and urea was indicated by an alkaline shift in pH while fermentation was indicated by an acid shift in pH.

Isolation of strains. Source and origin of specimens from which 10 strains were isolated are given in Table I. Two strains were isolated from brain tissues of two animals infected with scrapie, 1 from a sheep (C506) with naturally acquired scrapie and the other from a mouse (G230) with scrapie experimentally induced by the intracerebral injection of a brain suspension from another sheep with naturally acquired scrapie. The latter suspension was not available for direct culturing for *Mycoplasma*. These 2 *Mycoplasma* strains were isolated by direct culture in both BYE and Hayflick broth and agar media aerobically and anaerobically.

One strain (BBLG119) was isolated during a localized outbreak of suppurative arthritis affecting 15 goats in a herd of 60. Specimens were obtained from two affected goats and cultured for *Mycoplasma*. In one goat, the swollen joint was scrubbed clean, washed with thimerosal, and 4 ml of hemorrhagic exudate were aspirated. *Mycoplasma* strain BBLG119 was isolated in high titer from this exudate which contained 10^4 colony forming units (cfu)/ml.

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TABLE I. Source of Specimens and Media Used for Primary Isolation of *M. arginini* Strains.[a]

M. arginini strains	Specimen		Media used for primary isolation
	Source	Obtained from	(Ref.)
	Brain		
G230	scrapie infected mouse	J. A. Morris Bethesda	(1, 2)
C506	scrapie infected sheep	J. A. Morris Bethesda	(1, 2)
	Exudate		
BBLG119	knee-joint of arthritic goat	R. A. DelGiudice Baltimore	(3)
	Cell cultures		
BBL88	continuous, lymph node of lymphatic lymphoma patient (8) (uninoculated)	J. M. Trujillo Houston	(3)
B40(I9)	WI-38 subline, human lung (5) fibroblast (uninoculated)	H. Yamashiroya Chicago	(1–3)
B59(I5)	primary dog (beagle) kidney (uninoculated)	H. Yamashiroya Chicago	(1–3)
B60(I6)	primary dog (beagle) kidney (uninoculated)	H. Yamashiroya Chicago	(1–3)
527	EB-3 subline, Burkitt lymphoma (7) (uninoculated)	B. G. Young Bethesda	(1, 2)
539	KB subline, human carcinoma of nasopharynx (6) (uninoculated)	V. Saureno Boca Ratan	(1, 2)
BER	primary chimpanzee kidney, inoculated with serum from normal patient	L. F. Barker Bethesda	(1)

[a] Strain BER was isolated by L. F. Barker, Bethesda, and strains B40, B59, and B60 were isolated from common tissue simultaneously by H. Yamashiroya, Chicago, and the authors. The strains were designated B40, B59, and B60 by BBL and I9, I5, and I6 by NIH. Other strains were isolated by the authors.

Seven strains were isolated from seven cell cultures. Six of these 7 strains were isolated from cells sent to us for routine examination for the presence of *Mycoplasma*. These cells included primary dog and chimpanzee kidney cell cultures and continuous human WI-38 (5), KB (6), Burkitt (EB-3) (7), and lymphatic lymphoma (8) sublines.

Serologic procedures employed were growth inhibition (9) and plate immunofluorescence (3) tests.

Mice for pathogenicity tests were obtained from the general purpose colony of the Animal Production Section, NIH, and test materials were injected intracerebrally on the first or twenty-first day of life.

Results. Growth of strains. Ten *Mycoplasma* strains were isolated with use of Hayflick (1), BYE (2), and BBL (3) agar media (Table I) and they grew readily in broth media with peak population titers ranging from 10^7 to 10^9 cfu/ml. Strains produced slight turbidity in broth and a typical "fried-egg" colony morphology on agar media. When horse serum was omitted from media, no growth occurred. All strains gave positive arginine and negative glucose and urea metabolic reactions.

Hemadsorption. Strains BBL88 and G119 failed to hemadsorb (10) chicken erythrocytes. Other strains were not tested,

Serologic characterization. Serologic relatedness was established by use of immunofluorescence and growth inhibition tests. Positive reactions were produced when antisera prepared to strains BBL88, C506, and G230

TABLE II. Serologic Relationship between Strains of *M. arginini* as Determined by Immunofluorescence and Growth Inhibition Procedures.[a]

M. arginini strains	Immunofluorescence reactions (reciprocal of serum conjugate titer) (goat antiserum to strain BBL88)	Growth inhibition reactions [zone of inhibition with undiluted serum (mm)]		
		Rabbit antisera to strains		Normal rabbit sera
		G230	C506	
G230	320	3	4	none
C506	160	4	4	none
BBLG119	320	3	5	none
BBL88	640	4	5	none
B40(I9)	640	4	4	none
B59(I5)	640	4	4	none
B60(I6)	640	4	4	none
527	320	4	4	none
539	320	4	5	none
BER	640	4	5	none

[a] Appropriate negative controls were included in these studies. See Tables III and IV.

were tested with each of the 10 strains isolated. Immunofluorescence titers obtained with fluorescein-conjugated antiserum prepared in goats to strain BBL88 ranged from 1:160 to 1:640, and the zones of growth inhibition obtained with antisera prepared in rabbits to strains G230 and C506 ranged from 3 to 5 mm (Table II).

Serologic unrelatedness to known species of *Mycoplasma* was established by use of the same technique described in the previous paragraph. In cross serologic reactions, *M. arginini* strains failed to react with any of 53 known *Mycoplasma* strains, representing at least 27 established *Mycoplasma* species and also 8 serologically distinct, but, as yet, unclassified *Mycoplasma* species derived from 8 different animal species sources (Tables III and IV). These results indicate that *M. arginini* is unrelated to known species of *Mycoplasma* and that it represents a new species.

Pathogenicity for mice. Fifty 1-day-old mice and 20 12–14 g mice were inoculated intracerebrally with a culture of strain G230 containing 10^6 organisms/inoculum. Mice did not develop signs of illness during a 1-year observation period. Tissues were not examined for persistence of *Mycoplasma* or for gross or histologic changes.

Discussion. Data presented indicate that *M. arginini* represents a new species. Negative serologic reactions were produced with 53 strains representing at least 27 known *Mycoplasma* species. Serologic studies were not performed with two recently described species, *M. synoviae* (11) and *M. hyopneumoniae* [Switzer (12)] or *M. suipneumoniae* [Whittlestone (13)], but these 2 species differ from *M. arginini* in 3 respects: they cannot be grown on Hayflick, BBL, or BYE media; *M. synoviae* requires nucleic acid derivative (NAD) supplements for growth; and they are fermenters.

Most *Mycoplasma* species can be placed into one of three groups depending on whether they utilize either arginine (14, 15) or dextrose (fermenters), or urea (16, 17) for energy. The strains isolated in this study produced positive arginine reactions, and, thus, the species designation selected was *M. arginini*. The prototype strain shall be G230.

This study was undertaken originally to determine whether *Mycoplasma* were present in the brains of sheep and mice with scrapie and the brains of man with kuru and was prompted by similarities in the clinical manifestations of scrapie in sheep (18), goats (18) and mice (19), kuru in man (20), and *M. neurolyticum* disease in mice (21). Absence of *Mycoplasma* in the eight kuru brains which were examined by us and their infrequent occurrence in brains of sheep (2 of 19)

TABLE III. *Mycoplasma* Species Listed Gave Negative Reactions in the Immunofluorescence and Growth Inhibition Tests with Antisera Prepared to *M. arginini* Strains BBL88, C506, and G230.

Human origin	
M. pneumoniae, strain FH	
M. hominis, strains PG21	
DC63	
DC327	
V2785	
Botte	
M. fermantans, strain PG18	
M. salivarium, strains PG20	
B3	
M. orale, type 1, strains CH19299	
(*M. pharyngis*) 274	
4R	
M. orale, type 2, strain CH20247	
M. orale, type 3, strain DC333	
M. lipophilium, strain MaBy	
Mycoplasma sp. (unclassified), strain Navel	
Murine origin	
M. neurolyticum, strains PG28	
Type A	
M. arthritidis, strain PG27	
(*M. hominis*, type 2)	
M. pulmonis, strains PG34	
Negroni	
880	
Mycoplasma sp. (unclassified), strain Type C	
Commensals	
M. laidlawii, strains PG8	
PG9	
PG5	
Canine origin	
M. spumans, strain PG13	
M. canis, strain PG14	
M. maculosum, strain PG15	
Avian origin	
M. gallisepticum, strains PG31	
293	
H1344	
M. gallinarum, strain PG16	
M. iners, strain PG30	
M. anatis, strain 1340	
M. meleagridis, strain 17529	
Porcine origin	
M. hyorhinis, strains 7	
GDL	
M. granularum, strain 39	
Bovine origin	
M. bovigenitalium, strain PG11	
M. bovirhinis, strain PG43	
Mycoplasma sp. (unclassified) strain 188 (calf)	
Caprine origin	
Mycoplasma sp. (unclassified) strains KS-1 (ATCC15718)	
BBLG145	
189 (kid)	
UM30847 (goat)	

with scrapie are indications that *M. arginini* is probably not associated etiologically with kuru or scrapie. In addition, *M. arginini* was isolated in high titer from the joint exudates of an arthritic goat during an outbreak of suppurative arthritis. The role that *M. arginini* plays in the ecology of its host was not established, and is a subject of current studies.

The common occurrence of *M. arginini* in contaminated cell cultures obtained from separate and distant laboratories indicates that this agent is a widespread contaminant. Thus, *M. arginini* may assume current importance since it constitutes yet another common, troublesome adventitious agent of cell cultures.

Summary. A new serologically distinct species of *Mycoplasma*, designated as *M. arginini*, was isolated directly from sheep, mouse, and goat tissues and from cell cultures derived from human, chimpanzee, and dog tissues. The 10 *M. arginini* strains isolated were capable of metabolizing arginine but not glucose and ura and they grew readily in several broth and agar media commonly used for growth of *Mycoplasma*. Strains of *M. arginini* were related serologically to each other but were unrelated serologically to a least 27 established species of *Mycoplasma*. The principal current interest in *M. arginini* arises from its presence as a common contaminant in cell cultures. The role that *M. arginini* plays in the ecology of its hosts remains to be determined.

TABLE IV. *M. arginini* Strains C506 and G230 Gave Negative Growth Inhibition Reactions when Tested with Antisera Prepared to *Mycoplasma* Species Listed.

Human origin	Avian origin
M. pneumoniae, strain FH	*M. gallinarum*, strain PG16
M. hominis, strain PG21	*M. gallisepticum*, strains 293
M. fermentans, strain PG18	H1344
M. salivarium, strain PG20	*M. iners*, strain PG30
M. orale, type 1 strains CH19299	Porcine origin
278	*M. hyorhinis*, strain GDL
4R	*M. granularum*, strain 39
M. orale, type 2 strain CH20247	Bovine origin
Murine origin	*M. mycoides*, var. *mycoides*[a], strain V-5
M. neurolyticum, strain Type A	Caprine origin
M. arthritidis, strain PG27	*M. mycoides* var. *capri*[a], strains VOM
(*M. hominis*, type 2)	Connecticut
M. pulmonis, strain Negroni	Mexican
Commensals	California
M. laidlawii, strains PG8	*M. agalactiae*[a]
PG9	
Cell culture	
Mycoplasma sp. (unclassified), strain Davis	
Canine origin	
M. spumans, strain PG13	
M. canis, strain PG14	
M. maculosum, strain PG15	

[a] Growth inhibition and complement-fixation tests were performed using these reagents against *M. arginini* strains BBL88 and BBLG119 through the courtesy of Dr. J. J. Callis, Plum Island Animal Disease Laboratories, Greenport, New York.

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Acid Hydrolase Activity of Granulomatous Tissue in the Lathyritic Rat* (33352)

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The pathogenesis of the experimental connective tissue disease, lathyrism, and the mode of the resultant disruption of collagen metabolism has been the focus of much recent attention. Characteristically, lathyritic collagen shows a marked increase in soluble molecules and a decrease in mature, insoluble collagen. Numerous theories on the mechanism of this phenomenon have been proposed, but none at present is totally reconcilable with all of the data. Lathyrogens appear, at least in part, to exert their effect on connective tissues by inhibiting the production of covalent cross-links. Bornstein and Piez (1) have presented evidence that aldehyde intermediates are involved in intramolecular cross-link formation and have proposed that lathyrogens inhibit the process by which specific lysyl residues of the tropocollagen molecule are oxidatively deaminated to aldehydes. Page and Benditt (2) have shown that pig plasma amine oxidase, an enzyme believed to be similar to the oxidase functional in aldehyde formation in collagen, is competitively and reversibly inhibited by the lathyrogen β-aminopropionitrile (BAPN).

Although investigations have centered mainly on the inhibition of cross-link formation in the collagen of lathyritic animals, some attention has been paid to the catabolic aspects of the disease. Tanzer and Gross (3) have presented evidence that there is some degradation of insoluble collagen and have postulated the possible involvement of a protease in lathyrism. Holzmann *et al.* (4) have demonstrated a significant increase in serum catheptic activity in lathyritic rats, and it has been shown (5) that protease-induced alterations of tropocollagen yield a material that will no longer form cross-links. Dense granules in cartilage matrix of chick embryos, that are believed to be protein–polysaccharide complexes, have also been shown to disappear shortly after the injection of BAPN (6).

To further evaluate the degradative aspects of lathyrism, and to investigate the possible role of lysosomal enzymes in this process, the activities of five acid hydrolases, of the lysosomal type, were determined in granulomatous tissue from normal and lathyritic rats.

Methods. Subcutaneous granulomas were induced by implanting 2 or 3 polyvinyl plastic sponges (weighing 120 $\pm$ 5 mg) subdermally on the dorsum of male rats of the Holtzman strain (initial weight 140–150 g). Beta-aminopropionitrile (BAPN) was either incorporated in the diet at a level of 0.4%, or was administered by intraperitoneal injection (144 mg/day). There were 6–9 rats in each group, and the control rats were pair fed. The rats on the dietary BAPN, and their controls, were sacrificed on day 18, and

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