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AN ULTRASTRUCTURAL SURVEY OF DESMOSOMAL-MITOCHONDRIAL COMPLEXES IN THE LIVER OF SEVERAL SPECIES OF LABORATORY MAMMAL[1]

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ABSTRACT. The frequency of desmosomal-mitochondrial complexes was studied in the liver of a variety of laboratory mammals with transmission electron microscopy. Variation in the frequency of complexes within orders and among species ranged from one complexed mitochondrion for every desmosome counted in hamsters and mice, one to two in primates, one to 32 in guinea pigs, to none in rabbits, cats, dogs, and fetal rats. The morphology of desmosomal-mitochondrial complexes was similar, but not identical, among species. Complexes were widely dispersed in normal liver. The function of the complex is speculative.

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INTRODUCTION

Literature reporting the complexing of mitochondria and the spot demosome (macula adherens) is not extensive (Asmussen 1980, Bernstein and Wollman 1975, Sternlieb 1969). Except for references to such complexes in acute intermittent porphyrea (Asmussen 1980), methotrexate treated psoriatics (Horvath et al. 1977), cholestasis (Rassat et al. 1981), Morris hepatoma, and in rats after vinyl chloride and ethanol exposure (Miller et al. 1982), it appears that this normal structure in the liver has been related to experimental conditions and disease states only rarely. Freeze-fracture methods have been used to identify gap and tight junctions and less often spot desmosomes (Staehelin 1974, Goodenough 1980, Robeneck et al. 1979, Metz et al. 1979, Montesano 1980, Tice et al. 1975); a structure which might be a desmosome with attached mitochondrion has not been reported.

Examination of thin sections of liver from numerous untreated laboratory animals has revealed a wide variation in the frequency of desmosomal-mitochondrial complexes. (The shortened term "desmite" has been adopted here for the association of mitochondria with the tonofilaments of the spot desmosomes along the plasmalemma of adjacent hepatocytes.) Desmosomal-mitochondrial complexes (des-mites) in the livers of rabbits, cats, dogs, and fetal and neonatal rats, were extremely rare. In male guinea pigs, they were infrequent, while in rats they were abundant. Because of this variation, a study was undertaken to quantitate the relative frequency of desmites in hepatocytes of some common laboratory mammals and to compare their morphology.

METHODS AND MATERIALS

Data reported in this study were derived from observations of 98 individuals belonging to 12 species and four orders (Rodentia, Lagomorpha, Carnivora, and Primates) in the class Mammalia. All tissues were fixed by immersion in a solution containing both paraformaldehyde and glutaraldehyde (Russell 1972, Rosen et al. 1967), and were postfixed in osmium tetroxide in the buffer corresponding to the fixative. Tissues were embedded either in Epon 812 or Spurr after stepwise dehydration in ethanols and propylene oxide.

Liver was obtained from cats, dogs, and non-human primates by surgical biopsy. Ferrets, guinea pigs, cats, dogs, and non-human primates were under Nembutal anesthesia at the time of sampling. Mice were sacrificed by cervical dislocation, hamsters and Sprague Dawley rats by decapitation and

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TABLE 1

Ratios of desmosomes to desmosomes complexed to mitochondria in the liver of various species.

Order	Species	Variety	Sex*	Age	No. Animals	Desmosomes Per Des-mite	Mirror** Images	Comments
Rodentia	*Rattus norvegicus*	Long Evans Hooded	M	Adult	4	3.7	18%	
		Long Evans Hooded	PF	Adult	3	4.3	12%	
		Long Evans Hooded	PF	Adult	4	3.5	18%	thyroid***
		Sprague Dawley	F	Adult	4	2.6	26%	
		Sprague Dawley	PF	Adult	4	2.8	27%	
		Sprague Dawley		Fetal	7	†	—	
		Sprague Dawley		2d Neonatal	4	†	—	
		Sprague Dawley		7d Neonatal	4	—	—	
		Sprague Dawley		12d Neonatal	2	—	—	
		Sprague Dawley		17d Neonatal	1	6.5	20%	
	Mus musculus House mouse	Swiss Albino	F	Weanling	7	1.4	49%	
	Mesocricetus auratus Golden Hamster		PF	Adult	3	1.0	53%	
	Cavia porcellus Guinea Pig	Hartley	M	Adult	8	32.0	—	
Lagomorpha	*Oryctolagus cuniculus*	New Zealand Albino	M	Adult	2	†	—	
Carnivora	*Mustela putorius* Ferret		M	Adult	2	2.7	6%	
	Felis catus Domestic Cat		M/F	Adult	6	—	—	2 control 4 treated
	Canis familiaris Domestic Dog	Beagle	M/F	Adult	7	†	—	4 control 3 treated
Primates	*Macaca mulatta* Rhesus monkey		M/F	Adult	4	2.8	46%	
	Macaca arctoides Stumptail macaque		F	Adult	3	1.6	30%	
	Papio cyanocephalus Yellow Baboon		M	Adult	4	†	—	2 control 2 treated

*M = male, F = female, M/F = males and females, PF = term pregnant female.

**Desmosomes which involve a mitochondrion in each participating cell are mirror images (double).

***Thyroid is included here, since it is the tissue where complexes were first described.

†Equivocal demonstration of complexes.

Long-Evans Hooded rats and rabbits by asphyxiation with CO_2.

Tissues from cats, dogs, and non-human primates were from a study on perfluoro-chemical based artificial blood. Details are given in papers by Clark et al. (1974, 1975) and Miller et al. (1975, 1978). One specimen of human liver was evaluated, three times, each at different levels in a single block. This sample was kindly supplied by Dr. Cynthia Daugherty, Department of Pathology, Childrens Hospital, University of Cincinnati.

Plastic blocks were sectioned on a Porter Blum MT2-B microtome with a diamond knife, and thin sections were placed on naked copper grids and contrasted with uranyl acetate and lead citrate. Sections were examined with a Siemens 1A electron microscope. Counts of desmosomes were obtained from five adjacent grid spaces from each block sectioned. The whole grid space was counted, and only spot desmosomes were included. Only those desmosomes with filaments which clearly were adjacent to mitochondria were counted as complexes. Total number of desmosomes per five spaces, total number of complexed mitochondria, as well as the numbers of single and double events (mirror image complexes) were tallied.

Eighty of the 98 animals examined morphometrically are given in table 1 which represents the results of counting of 400 grid spaces and a total of 4,450 desmosomes.

One-way analyses of variance were performed on the occurrence of desmites in adult rats, hamsters, mice, and macaques comparing (1) the frequency of desmosomes complexed with one mitochondrion (single); (2) number of desmosomes with mirror image complexes (double), and (3) the total number of complexes (des-mites). When overall ANOVA's were significant, individual group comparisons were made with *t*-tests and Duncan Multiple Range tests.

RESULTS

Desmosomes of adjacent hepatocytes in rats were as has been described in the literature. Other species differed from the rat in the number of desmosomes found per membrane distance and in the degree of development. Number of desmosomes counted per animal was an indicator of the relative frequency with which non-complexed desmosomes were found in liver since totals represent a constant area (five adjacent grid spaces of a 200-mesh grid). Rabbit liver contained many desmosomes, approximately 100 per grid space; rat liver 55, hamster 50, mouse 40, dog 35, ferret 35, primate 30, guinea pig 28, and fetal rat 25. Though not absolute, these numbers demonstrate a relative difference in the occurrence of spot desmosomes. As seen in table 1 the number of spot desmosomes in a grid space was not necessarily related to the number of desmosomes which were complexed with mitochondria (des-mites).

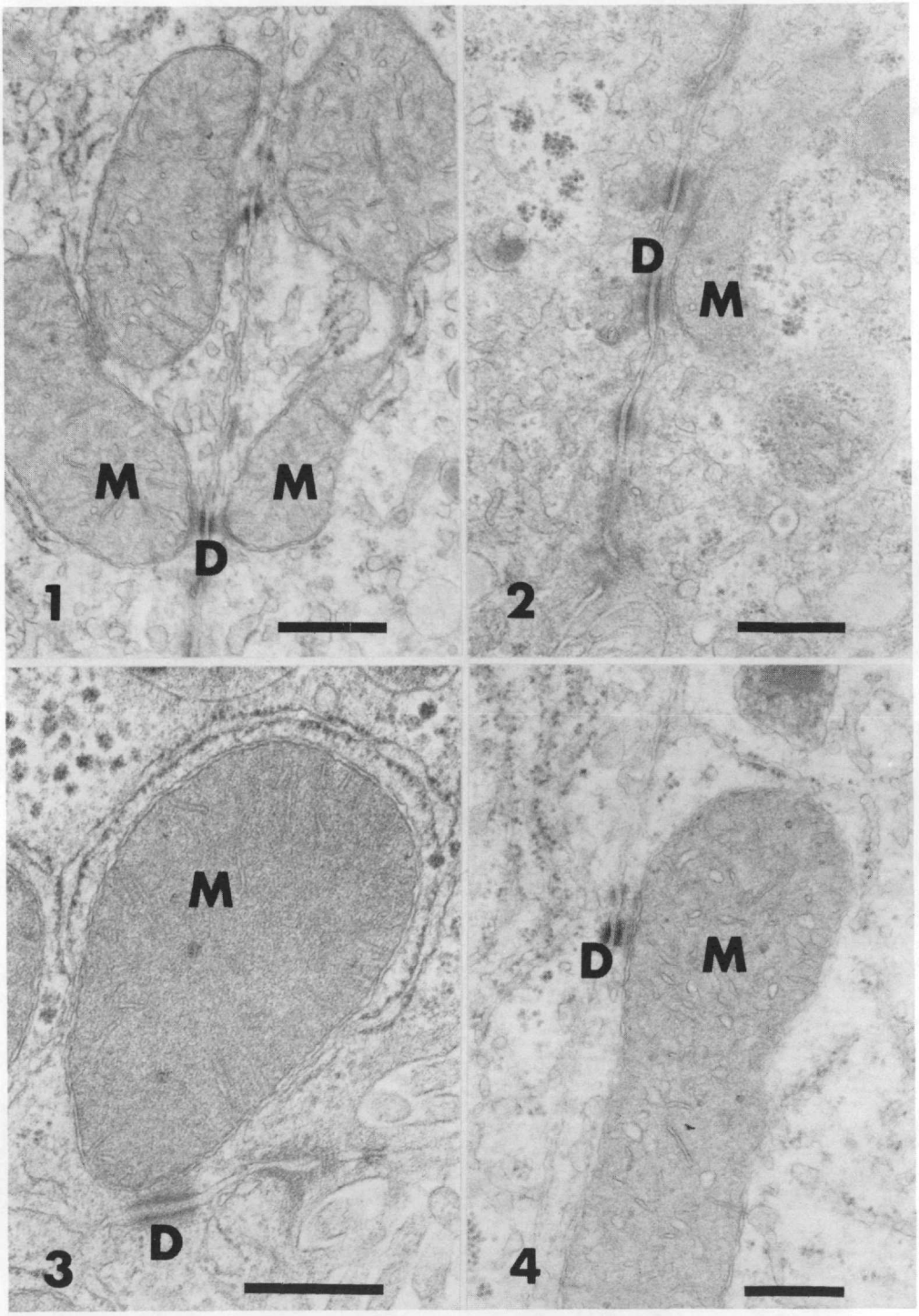
Des-mites were clustered in all species (fig. 1, 2, 5, 8, 10). Two or three complexes could be found along the plasmalemma of adjacent hepatocytes, at the sinusoidal and bile canalicular borders. They were most frequently identified just beneath the junctional complexes adjacent to the bile canaliculus. Mitochondria which participated in the complexing were often distorted, giving the impression of being pulled over to the desmosome by the looping tonofilaments forming part of the intracellular plate of the desmosome (figs. 3, 5, 13). Outer membranes of mitochondria were not noticeably thickened, nor were the tonofilaments attached in such a way as to ruffle the membrane

FIGURE 1. Adult rat hepatocytes have many desmosomes with complexed mitochondria which occur in clusters. Mitochondria are misshapen and "pulled" at points of contact with the tonofilaments of the desmosome. Mitochondria, M; desmosome, D; bar, 0.5μ 29,845×.

FIGURE 2. Opportune sections through several complete desmosomes in the rat liver demonstrate clustering. More than one desmosome can be complexed with a single mitrochondrion. Mitochondrion, M; desmosome, D; bar, 0.5μ 31,725×.

FIGURE 3. Des-mites in the liver of the ferret are similar in structure to those in the hamster, rat, mouse and macaque, though total number of des-mites and the ratio of single to double (mirror image) des-mites is quite different. No alteration in the outer membrane of the mitochondrion is seen. Mitochondrion, M; desmosome, D; bar. 0.5μ 39,104×.

FIGURE 4. Adjacent hepatocytes from a 17 day old neonatal rat are joined by a desmosome with complexed mitochondrion. In animals younger than 17 days, and in term fetuses, complexes of desmosomes and mitochondrian complexes were extremely rare, and poorly defined. Mitochondrion, M; desmosome, D; bar, 0.5μ 29,469×.


M
M
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1
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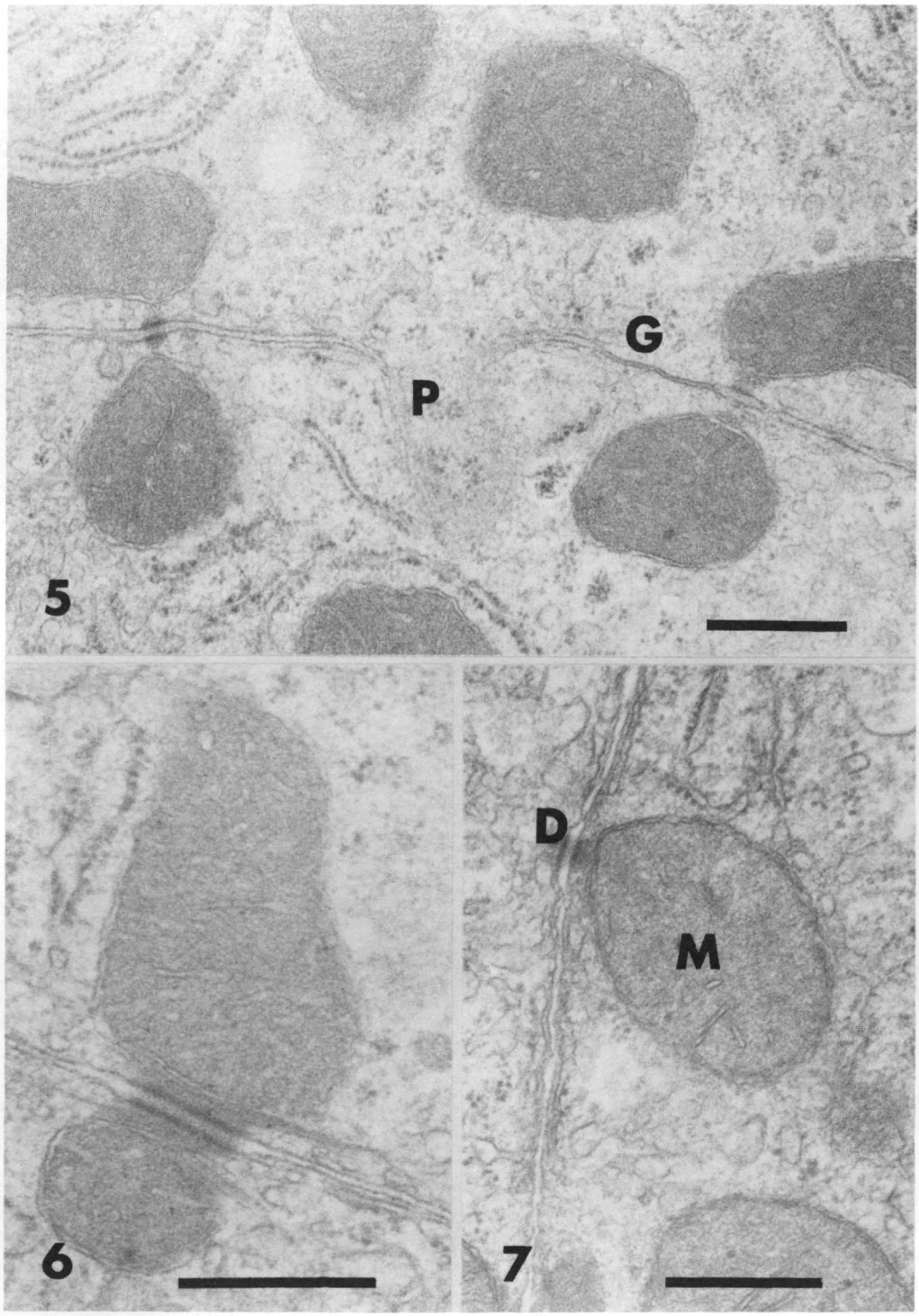
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(figs. 1-15). In fact, areas of flattening were often seen (figs. 3, 6, 11, 13) which may represent sections through central areas of the complexed spot desmosome. On some opportune sections a periodicity was seen at the outer membrane, most likely representing cross sections of the adjacent tonofilaments (fig. 12). In many species, the desmites were found adjacent to other membrane specializations such as gap (communicating) junctions (figs. 5, 8, 13). In the mouse, in particular, where multiple attachments were seen between mitochondria and desmosomes, desmosomes appeared to be in different stages of development. This can be visualized in fig. 10, where three junctions are observed with two mitochondria. The complete desmosomes, with a slight narrowing of the intercellular space, central dense line, and intracellular dense plates and tonofilaments, seemed unaltered by the presence of complexed mitochondria. In addition, mitochondria which were in some way unusual, e.g. those with parallel cristae, paracrystalline inclusions or floccular densities, were not seen complexed with desmosomes. The association of the two mitochondria (mirror images, doubles), one within each of the cells participating in the desmosome, was extremely frequent (table 1; figs. 1, 2, 5, 6, 8, 11-14). The area of contact between mitochondrion and desmosome was often extensive (figs. 3, 6, 12, 14), especially in hamsters.

Macaca mulatta and *M. arctoides* demonstrated large, well-developed desmites, which resembled those of rodents in general (figs. 13-15). Doubles were frequent in primates but significantly fewer than in the mouse or hamster. Except for *Papio cyanocephalus*, des-mites in the primate liver were plentiful (human included). As in rodents, they occurred at the bile canaliculus beneath the typical junctional complexes, and also at the sinusodial border. Guinea pigs had very few, poorly developed des-mites. The occurrence of des-mites was considerably less in dogs, cats and rabbits than in mice and hamsters (table 1).

Fetal rat liver provided only rare examples of desmosomal mitochondrial complexes. Des-mites began to appear in the neonatal animal about d 17 of age and were similar morphologically to those of the adult rat liver (fig. 4). An inverse relationship was observed between the number of hemopoietic cells in the fetal and neonatal liver and the increase in the incidence of desmosomes and des-mites.

One-way analyses of variance indicated that species had a significant effect on all three dependent variables (single, double, and total des-mites). Hamsters and mice were not significantly different from each other in the three parameters analyzed. Mice and hamsters were, however, significantly different from macaques in all parameters and from rats in all parameters except in the number of desmosomes with a single complexed mitochondrion ($p < 0.07$). Statistical analyses were not performed on guinea pigs, rabbits, dogs and cats or fetal rats where des-mites were rare or unobserved.

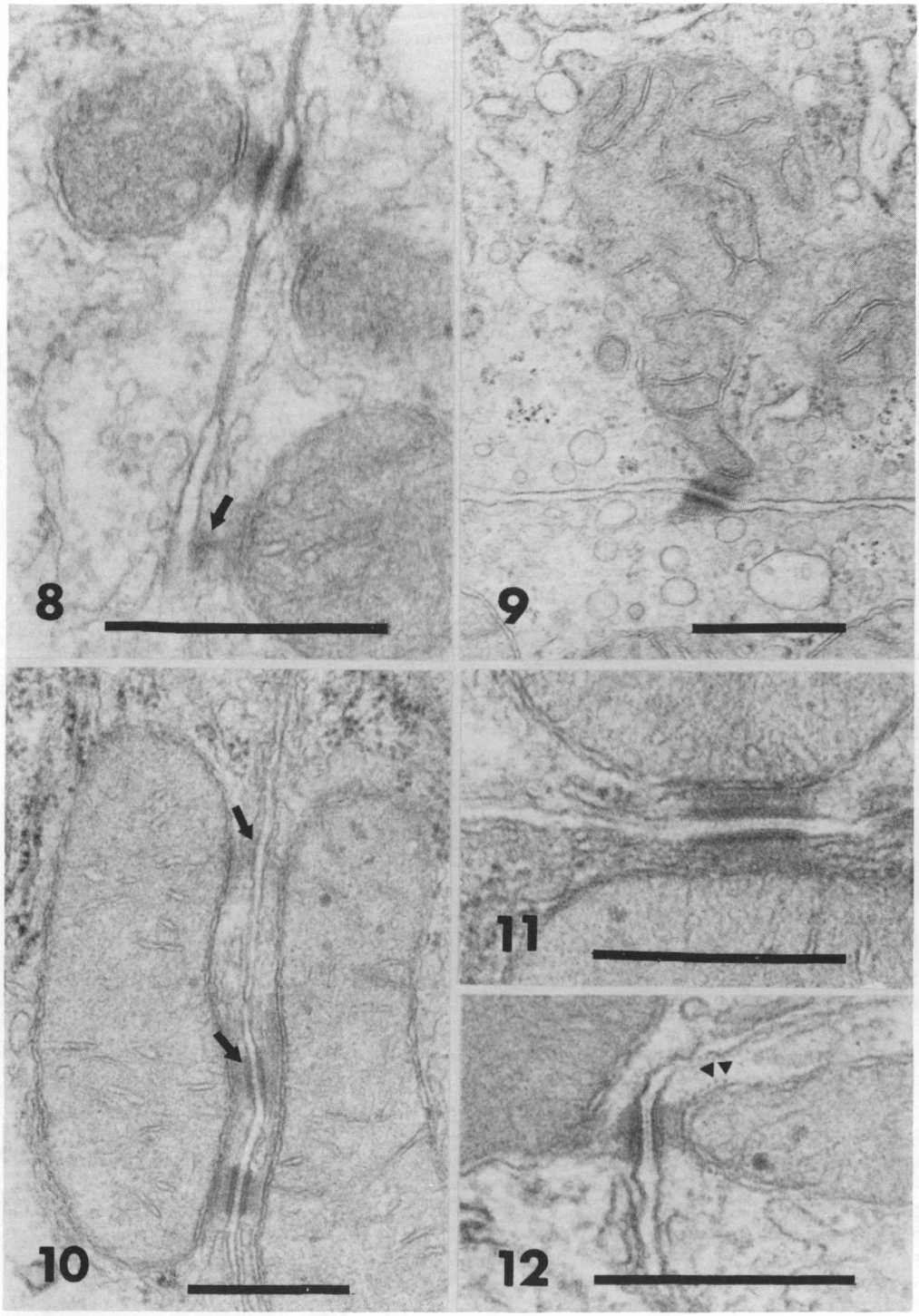
DISCUSSION

Desmosomes have been described in the literature as specializations of two parallel plasmalemmae containing an intercellular density with a prominent central dense

FIGURE 5. Hepatocytes in the hamster display numerous complexes both single and mirrored. They are frequently seen near other membrane specializations, e.g. pegs and holes (P), and gap junctions (G). Distortion of the mitochondrion toward the desmosome is also seen. Bar, 0.5μ 39,480×.

FIGURE 6. An extensive attachment and large area of tonofilaments is seen in this double complex in hamster liver. Bar, 0.5μ 58,280×.

FIGURE 7. Desmosomes in the hamster liver show a prominent outer leaflet of the plasma membrane giving an illusion of three separate intercellular densities. To a lesser extent, guinea pig and rabbit show this also. Desmosomes with complexed mitochondria vary in appearance. Mitochondrion, M; desmosome, D; bar, 0.5μ 45,120×.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line, and an intracellular dense plaque with gently arching tonofilaments (Fawcett 1981, Staehlin 1974). In the present study there was slight variation in the size of non-complexed desmosomes among species, and in the prominence of the outer leaflet of the plasmalemma, which was more pronounced in hamsters, guinea pigs and rabbits. Many reports of tight and gap junctions utilizing freeze-fracture have been published, but fewer studies have been published on spot desmosomes (Staehelin 1974, Metz et al. 1979, Montesano et al. 1975). Desmosomes are primarily a modification of the inner aspect of the cell membrane, and are not apt to be seen in the preparations unless the desmosome is cross-fractured. Apparently such preparations have not revealed any complexed mitochondria.

The spot desmosome was the only junctional specialization which was visualized with adjacent mitochondria. Both desmosomes and desmosome-mitochondrion complexes have been noted in epithelia with great regularity (Sternlieb 1969). They have been in the thyroid gland (Bernstein and Wollman 1975), in human liver (Asmussen 1980, Rassat et al. 1981, Sternlieb 1969), and in rat liver (Miller et al. 1982).

The number of desmosomes which were complexed to mitochondria in hepatocytes varied among species. Larger, more conspicuous des-mites occurred in macaques, rats, mice, and hamsters. Fewer and less prominent des-mites occurred in other species. Given the paucity of desmosomal-mitochondrial complexes in some species, it was difficult to determine whether real differences in the size of des-mites occurred or whether peripheral sections were encountered. The numbers of desmosomes complexing with mitochondria on adhering surfaces of the adjacent cells (doubles, or mirror images) also differed among species. Half of the des-mites were mirrored in hamster and mouse, while primates, rats and ferrets had significantly fewer (table 1). Sternlieb (1969) estimated double events to occur about 33% of the time in human liver which is similar to that which we obtained in macaques (table 1).

Des-mites occurred in groups, or clusters, sometimes with one mitochondrion complexed with three desmosomes. This provides circumstantial evidence for mitochondrial involvement in the modification or formation of desmosomes along a membrane. Changes in the desmosomal connections on a plasma membrane, or removal of desmosomes may be considered a normal physiological function. The frequency of des-mites in the liver was not consistent within a phylogenetic taxon. They were numerous in the primates studied except the male baboon. In the order Carnivora, des-mites were frequent in ferrets but were equivocally identified in dogs and not seen at all in cats (table 1). Rodents contained numerous des-mites,

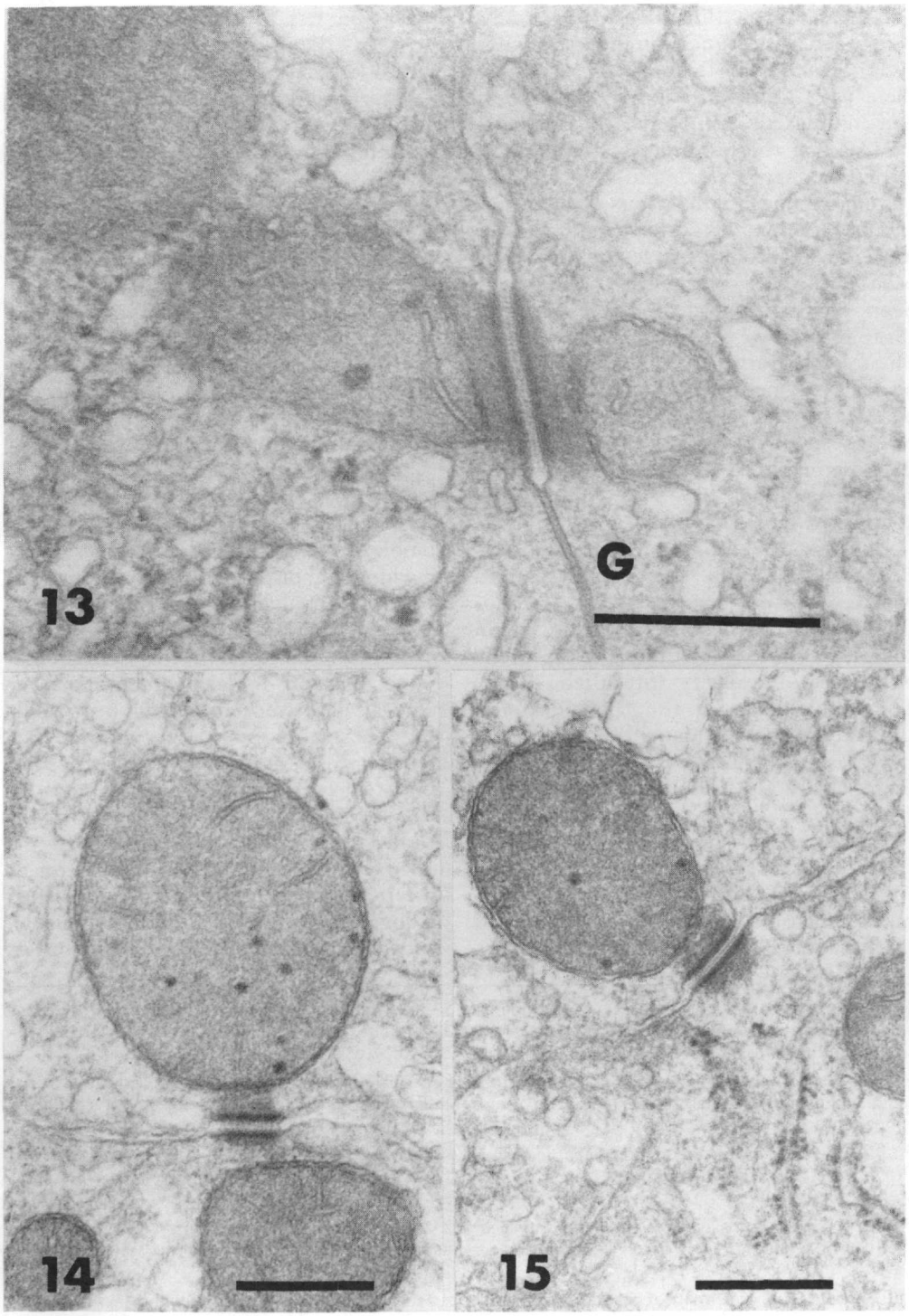
FIGURE 8. Des-mites in hepatocytes in the mouse are seen adjacent to gap junctions, frequently in clusters, and occasionally in the unusual association seen here. The mitochondrion is adjacent to a density (arrow) which is not a typical hemi-desmosome. Bar, 0.5μ 83,331×.

FIGURE 9. In hepatocytes of the mouse and all other species, reaching of the mitochondrion toward the tonofilaments of the desmosome is observed. The alignment of cristae within the complexed mitochondrion is not unusual. Bar, 0.5μ 38,399×.

FIGURE 10. These mirror image or double des-mites observed in this micrograph of mouse liver suggest that mitochondria participate in the assembly and/or degradation of this structure. Immature desmosomes, arrows. Bar. 0.5μ 47,470×.

FIGURE 11. An extensive area of contact between mitochondria and desmosome (mirror image) is seen in the liver of this mouse. No involvement of the outer membrane of the mitochondrion is seen. Bar, 0.5μ 73,696×.

FIGURE 12. A mirror image des-mite from the liver of a mouse shows the outer mitochondrial membrane, and cross section of adjacent tonofilaments (arrowheads). Bar, 0.5μ 73,696×.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except for guinea pigs, where they were infrequent. Lagomorphs (rabbits) demonstrated none, though the sample was small. An absence of des-mites in the liver of any species examined in this study is not implied; rather, the results suggest their relative frequencies.

The rarity of des-mites in fetal and early neonatal rat liver is consistent with the published literature. Studies of fetal human liver (Zamboni 1965), developing chick liver (Stephens and Bils 1967), fetal rat liver (Wood 1965, Luzzatto 1981) and neonatal liver obtained immediately after birth to 23 d postnatal (Jezequel et al. 1965), failed to identify the desmosomal-mitochondrial complexes. Though these studies were not focused on identification of this structure per se, des-mites were not seen in the micrographs which accompanied their works.

The assembly of the desmosome was proposed by Staehelin (1974) as a localization of masses of slightly dense cytoplasmic material on intracytoplasmic surfaces of adjacent epithelial cell membranes. An intercellular density appears and then an intercellular dense line. Dense plates and sweeping tonofilaments are formed on the cytoplasmic side of the plasmalemmae. Mitochondria have been observed complexed with desmosomes in comparable stages in adult mouse liver (fig. 10). Since more mitochondria appear to be complexed with developing desmosomes (figs. 1, 4, 7, 8, 10, 14) than with larger, completely developed desmosomes (figs. 6, 11, 13), complexing may be necessary during synthesis and/or degradation.

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FIGURE 13. The desmosome-mitochondrion complexes from *M. mulatta* are well developed, numerous and often double. The contacts between tonofilaments and mitochondria are wide. This particular des-mite is adjacent to gap junction. Gap junction (G); bar, 0.5μ 66,176×.

FIGURE 14. The des-mites of the liver of *M. arctoides* resemble closely those of *M. mulatta*. Contacts are extensive, and well developed. Mirror images are common. Bar, 0.5μ 42,394×.

FIGURE 15. No abnormal mitochondria were observed complexed (associated) with desmosomes in any of the tissues studied. Primates and dogs, for example, had many mitochondria with parallel cristae but none were involved in complexes. The liver of *M. arctoides* is seen here. Bar, 0.5μ 40,589×.

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