STUDIES ON THE PATHOGENESIS OF RABIES IN INSECTIVOROUS BATS*

I. Role of Brown Adipose Tissue

By S. EDWARD SULKIN, Ph.D., PHILIP H. KRUTZSCH,‡ Ph.D., RAE ALLEN, and CRAIG WALLIS

(From the Departments of Microbiology and Anatomy, The University of Texas Southwestern Medical School, Dallas)

(Received for publication, April 27, 1959)

Rabies, a disease known from antiquity, has received much attention by numerous investigators yet little is known regarding the survival mechanism for the virus in nature. The generally held concept that rabies is invariably fatal once symptoms become established in an animal may require revision. There is accumulating evidence that certain animals (lemmings, voles, ground squirrels), including man, may be subject to subclinical rabies (1, 2). Furthermore, the existence of symptomless carriers among animals shown to be transmitters of the disease would provide an important link in the biological chain of events. It has been clearly established that the vampire bat (*Desmodus rotundus murinus*, Wagner) and perhaps other species of bats serve as symptomless reservoirs for the virus in nature (3–5).

The existence of symptomless carriers of the rabies virus was first demonstrated with the discovery that vampire (hemophagous) bats in some sections of Mexico and South America may be infected with rabies. The history of rabies in the vampire bat and in other species of bats in South and Central America and in Mexico has been reviewed by Enright (6). Current interest in the public health significance of bats in rabies results from the demonstration of the rabies virus in many species of insectivorous bats collected in numerous sections of the United States. Since the initial demonstration in 1953 of the rabies virus in a lactating Florida yellow bat (*Dasypterus floridanus*) in Florida by Venters and his associates (7) and the almost simultaneous isolation of the virus from the colonial cave-dwelling Mexican free-tailed bat (*Tadarida mexicana*) in Texas by Sullivan and her colleagues (8) and by Burns and Farinacci (9), at least 11 species of colonial and 5 species of non-colonial bats collected in 18 states have been found to be infected with the rabies virus (10, 11). Similar observations have also been made in Canada, Yugoslavia, and Hungary (10, 12).

* This investigation was supported in part by Research Grant (E-2316) from the National Institutes of Health, United States Public Health Service, Bethesda, and by a grant from the Caruth Foundation, Dallas.

‡ Present address: Department of Anatomy, University of Pittsburgh School of Medicine, Pittsburgh.

These surveys of bat populations for the presence of rabies virus were prompted by numerous human exposures to bats shown to be infected (7, 13–15). In all such cases reported the individuals bitten by rabid bats received Pasteur treatment and no deaths occurred. However, at least three human rabies deaths are believed to have resulted from exposure to bats in this country. The first, reported by Sulkin and Greve (16), concerns a woman who died of rabies in October, 1951, 25 days after an encounter with a bat. The second, a member of a team of the entomology section of the Texas State Department of Health conducting bat rabies investigations, occurred in January, 1956 (15). The circumstances of these two cases were such that the bats involved could not be examined for rabies infection. The third fatal case was a woman who died of rabies 66 days after an encounter with a bat proved to have been rabid (17). Rabies developed in this patient despite prompt use of combined seroprophylaxis and duck embryo rabies vaccine. The demonstration of rabies virus in several species of bats together with the increasing number of proved exposures to rabid insectivorous Chiroptera, would suggest that these animals may provide a missing link in the natural history of the disease.

The demonstration of lipotropism in cortisone-treated hamsters infected with poliovirus by Shwartzman (18, 19) and the reported invasion of brown fat of suckling mice infected with certain strains of Coxsackie virus by Pappenheimer *et al.* (20), Dalldorf (21), and Godman *et al.* (22) suggested an approach to the study of the role of Chiroptera in the biological life cycle of the rabies virus. Reproducible histologic lesions and high infective virus titers in brown adipose tissue in cortisone-treated hamsters infected with poliovirus have been observed, indicating marked lipotropism in this infection (23). These observations have been confirmed in part by Bodian (24) who showed high concentrations of poliovirus in brown fat in chimpanzees after the viremic phase of the infection. This investigator suggested that the brown fat might be regarded as a "target organ" or secondary site of poliovirus multiplication. It has also been suggested that the susceptibility of this tissue to certain virus infections is so marked as to persist when most other organs and tissues have become refractory (20). Preliminary studies in this laboratory (25) have already indicated that the brown adipose tissue of the Mexican free-tailed bat (*Tadarida mexicana*) may provide a depot for storage of the rabies virus.

The purpose of the present study has been to examine the progress of rabies infection in experimentally infected insectivorous bats with the view to learning something of the possible mechanism by which these animals may serve as persisting reservoirs for the virus in nature. Two species of bats, the Mexican free-tailed bat (*Tadarida mexicana*) which is a quasi hibernator and the little brown myotis (*Myotis lucifugus*), a true hibernator, were used for the purpose of locating anatomical sites of rabies virus multiplication. In planning these studies we adopted as a working hypothesis the proposition that those tissues which provide food reserve during the inactive state (or hibernation) may also provide the appropriate means for storage of the virus during the period of

latency. Since there is considerable evidence that the interscapular brown fat is an actively metabolizing tissue serving as a source of food material during winter sleep, special emphasis is placed on the possible role of this tissue in the pathogenesis of rabies in these animals.

Materials and Methods

Virus.—Rabies virus, designated Thompson strain, isolated in albino Swiss mice from a human brain and in its second intracerebral passage in white mice was used in this study. The LD_{50} titer of the stock virus suspension for 3-week-old white Swiss mice inoculated intracerebrally was $10^{-4.8}$. Diluent for virus inoculum consisted of 10 per cent rabbit serum in physiological salt solution containing 250 units of penicillin and 250 μg. of streptomycin per ml.

Mice.—Three to 4-week-old white Swiss mice (CFW) obtained from two animal breeders were used throughout these studies.

Hamsters.—Six-week-old and suckling hamsters obtained from the Lakeview Hamster Colony, Newfield, New Jersey, were used in these studies. The animals were inoculated intramuscularly into the hind limb; the inoculum in the 6-week-old hamsters consisting of 2,000 mouse i.c. LD_{50} in 0.1 ml. and 800 mouse i.c. LD_{50} in the suckling animals.

Bats.—Two species of insectivorous bats were used in these experiments. Mexican free-tailed bats (*Tadarida mexicana*) were obtained from the Blowout Cave, Blanco County, Texas.[1] No infected bats were found in this cave in surveys conducted within the limits of the Edwards Plateau, an extensive tableland composed chiefly of cretaceous limestone. The little brown myotis (*Myotis lucifugus*) was obtained in northern West Virginia and southwestern Pennsylvania.[2]

The Mexican free-tailed bats had been netted on the day experiments were initiated. The little brown bats were usually inoculated 2 days after they were collected. The handling of bats during inoculation and transfer from field to laboratory cages was facilitated by a short period of inactivation at 4°C. Animals were inoculated by various routes: intramuscularly (i.m.) into the heavy muscle over the chest, into the surgically exposed interscapular brown fat (b.f.) and into the intrascapular area. In each instance the dose was approximately 8,000 mouse intracerebral LD_{50} in 0.1 ml.

The inoculated animals were placed in specially designed cages and maintained at 29°C. ± 2 (relative humidity 65 per cent). The bats were fed a mixture of 1 pound of large curd cottage cheese, 1 large banana, 1 ounce of codliver oil, and approximately 150 mealworms homogenized in a Waring blendor. Food and water were provided daily. A detailed description of the methods used for the safe laboratory care and successful maintenance of experimentally infected bats has been described elsewhere (26). All animals were observed several times daily during the observation period, and those which died during the 1st week after inoculation were discarded and not included in the analyses. Studies dealing with the laboratory maintenance of these animals have indicated that those which survive 7 or 8 days in captivity can usually be kept for several months (26). Throughout the course of the experiment the animals were observed for signs and symptoms of rabies infection. To limit the potential hazard involved in working with these animals all personnel engaged

[1] We should like to acknowledge free access to the Blowout Cave (Blanco County, Texas) granted by Mr. Gould Davis, rancher and owner, without whose cooperation this study could not have been completed. We also wish to acknowledge the assistance of Mr. William Hanszen, rancher, who helped in netting bats.

[2] The authors are indebted to members of the Pittsburgh Grotto of the National Speleogical Society for assistance in netting these bats.

in this study were immunized with modified live virus rabies vaccine of chick embryo origin (Lederle).

Preparation and Assay of Tissues.—The selective affinity of various tissues of the bat for rabies virus was shown by viral assay in 3- to 4-week-old white Swiss mice. Tissues were removed, following etherization and exsanguination, from apparently healthy animals as well as from animals with symptoms suggestive of rabies. In a few instances tissues were collected from animals which had been dead a very short time. Brain, salivary gland, and interscapular brown adipose tissue were collected as aseptically as possible using separate instruments for each specimen to avoid cross-contamination. In the experiments with hamsters a muscle biopsy at the site of the inoculation and kidney were also tested for virus. Since Negri bodies are not always demonstrable in tissues of bats proven to contain virus by mouse inoculation (27, 28) histologic examination of the various tissues was not made as routine. Tissues were ground in a mortar with sterile alundum and rabbit serum-saline diluent to make 5 to 10 per cent suspensions of brain, brown fat, and salivary gland. For qualitative assay 3 to 5 mice were inoculated intracerebrally with each specimen and observed for a 21 day period before being discarded as negative. In doubtful instances mouse brains were examined histologically and subinoculated into additional animals. In the quantitative assays serial 10-fold dilutions of the tissue suspensions were inoculated intracerebrally into groups of 5 mice each and the LD_{50} titer was established in each instance by the method of Reed and Muench (29).

RESULTS

Experiments with the Mexican Free-Tailed Bat (Tadarida mexicana)

Mexican free-tailed bats were used because of the tremendous populations of this species of Chiroptera in the southwestern part of the United States, and because a large proportion of the rabies virus isolations have been from this species (11). An important consideration in planning these studies was the selection of the strain of rabies virus to be used. Since rabies viruses isolated in nature seem to have different tissue tropisms, as evidenced by their varying ability to invade and multiply in the salivary gland (1), it seemed desirable to select a highly virulent strain known to produce a widely disseminated infection. In the initial phases of these studies, a strain of rabies virus recovered from the brain of a Mexican free-tailed bat, kindly provided by Dr. J. V. Irons, was used. However, this virus strain exhibited variable incubation periods and following only a few serial passages in both bats and mice became virtually avirulent for these hosts. A strain of canine street virus, isolated in this laboratory from the brain of a fatal human case and shown to be uniformly fatal for weanling hamsters following intramuscular injection with subsequent isolation of virus from brain, salivary gland, brown fat, and kidney, was used in these studies. In preliminary experiments (25) qualitative assays for virus in bats inoculated intramuscularly with rabies virus showed evidence of infection in 24 per cent of the animals during the observation period of 3 months. Virus was detectable in the brain, salivary glands, and interscapular brown fat. On the basis of quantitative assays in mice these preliminary data also showed that virus is most readily detectable in this host between the 20th and 30th day following intramuscular inoculation.

This initial experiment provided evidence that the brown adipose tissue may serve as a depot for the storage and perhaps multiplication of virus and stimu-

lated us to extend these studies to include larger numbers of animals for more precise observations. The results of these experiments are summarized in Table I.

TABLE I

Demonstration of Rabies Virus in Interscapular Brown Adipose Tissue and Other Tissues of Bats (Tadarida mexicana) Inoculated Intramuscularly

Exp. No.	No. infected / No. inoculated*	Per cent infected	Time after inoculation‡			Rabies virus demonstrated in§		
			Dead < 4 hrs.	Sacrificed with symptoms	Sacrificed healthy	Brown fat	Salivary gland	Brain
			days	*days*	*days*			
1	32/137	23.8	22, 42	22		+	+	+
			22	26	22, 25	+	−	+
					32, 32, 75, 75	+ [34.4]	− [31.2]	− [87.5]
			22, 25, 51	22, 26	50, 51	−	+	+
			22–35 (10)	26, 66	30, 35	−	−	+
2	41/215	19.1	28		22	+	+	+
				26		+	+	−
			24, 59		13	+	−	−
					20	+ [17.1]	− [34.1]	+ [80.5]
			24, 36‖, 40	26–41 (5)	21	−	+	+
			18–34 (6)	18–35 (5)	15–41 (11)	−	−	+
			25, 52, 59			−	+	−
3	31/140	22.1	19, 30			+	+	+
					26	+	−	−
			28, 60			+ [16.1]	− [38.7]	+ [93.5]
			22–38 (5)	30, 31, 59	41	−	+	+
			21–30 (5)	24,30,38	15–30 (6), 76, 83	−	−	+
			28			−	+	−

* Virus inoculum approximately 8,000 mouse intracerebral LD_{50}. Animals which died during 1st week after virus inoculation are not included in the analysis.

‡ Figure in parentheses refers to number of bats.

§ Figure in brackets indicates per cent positive among animals shown to be infected.

‖ Bat salivary gland virus also recovered from the salivary gland.

The number of Mexican free-tailed bats used in each of three separate experiments is indicated; those which died during the 1st week after virus inoculation are not included in these summaries. Each bat received an intramuscular injection into the heavy muscle over the chest of approximately 8,000 mouse i.c. LD_{50} contained in 0.1 ml. The animals were observed frequently for signs and symptoms of rabies. Close inspection of each animal was difficult because of the obvious risk to the investigator and the danger of releasing infected animals. Symptoms, when observed, consisted of irritability and aggressiveness, and occasionally paralysis. Some animals were sacrificed in apparent good health. Only tissues from animals which had been dead less than about 4 hours were assayed for rabies virus because virus is destroyed in tissue undergoing autolysis.

It can be seen that the infection rate in each of the three experiments is essentially the same (23.8, 19.1, and 22.1 per cent, respectively). The frequency with which virus was demonstrated in the brown adipose tissue varied somewhat. In Experiment 1

virus was demonstrated in this tissue in 34.4 per cent of animals shown to be infected, while in experiments 2 and 3 virus was demonstrated in brown fat in 17.1 per cent and 16.1 per cent, respectively, of infected animals. However, the frequency with which virus was demonstrated in the salivary gland (31.2, 34.1, and 38.7 per cent, respectively), and in the brain (87.5, 80.5, and 93.5 per cent, respectively) did not vary significantly in each of the experiments. The noticeable difference in the number of virus isolations from the brown fat in Experiment 1 as compared with Experiments 2 and 3 may be related to the season of the year when the bats were netted. Experiment 1 was carried out in the autumn when these migratory animals are preparing for their annual southward migration, and at a time when the interscapular brown fat lobes are large. These animals undergo considerable loss of weight between the time they leave the caves in the late fall and return in the spring (30). The other two experiments were initiated in the early spring after the animals have returned to the caves and when the amount of brown fat tissue available for virus assay is at a minimum. The bat salivary gland virus was encountered in only one instance (31, 32). A dual infection was demonstrated in a bat which was found dead 36 days after inoculation with rabies virus. Both viruses were recovered from the salivary gland of this animal.

In the experiments just described rabies virus was demonstrated in the brown fat in 23 of 104 animals shown to be infected following intramuscular inoculation. To establish whether or not virus actually multiplies in the brown fat, various tissues from these animals were titrated for virus content. The results of these observations, summarized in Table II, indicate that virus is widely distributed in this host between the 20th and 40th day, and occasionally as long as the 60th day, following intramuscular inoculation. Also there seems to be more active viral proliferation during this period of the infection. Among the animals sacrificed without visible signs or symptoms of rabies, virus titers in the brain ranged from $10^{-2.5}$ to $10^{-3.5}$, while in the brown fat of these animals only an occasional animal had levels in this tissue above $10^{-1.5}$. Virus was present in the salivary gland of only two of these animals; in each instance the titer was $10^{-3.0}$. Generally the levels of rabies virus were higher in those animals found dead or those sacrificed when symptoms were observed. Virus titers in the brain of some of these animals reached levels of $10^{-3.7}$. Virus concentrations in the salivary gland and brown fat were somewhat lower. In two instances the titer in the brown fat was $10^{-3.0}$. These data indicate that viral multiplication actually occurred in the brown adipose tissue, as well as in the salivary gland and brain of some of these animals, but the virus concentration even in the brain never did reach the level of the stock virus suspension ($10^{-4.8}$) used in initiating the experiments. Significant virus levels were demonstrated in the brown fat and salivary gland of an animal which developed symptoms of rabies 26 days after intramuscular inoculation of virus. Failure to demonstrate virus in the brain of this animal may have been due to autosterilization (33). In several instances low levels of virus were detectable in brown fat tissue

TABLE II

Levels of Rabies Virus in Interscapular Brown Adipose Tissue, Brain, and Salivary Gland of Mexican Free-Tailed Bats (Tadarida mexicana) Inoculated Intramuscularly

Condition of bats	Time after inoculation*	−Log LD_{50} in mice (i.c.)		
		Brown fat	Salivary gland	Brain
	days			
Dead < 4 hours	19	2.0	2.5	3.7
	22	1.66	2.33	2.43
	22	<1.0	−	2.75
	24	1.5	−	−
	28	2.5	<1.0	3.2
	28	1.5	−	3.7
	30	3.0	2.5	3.5
	42	2.0	2.7	3.0
	59	2.0	−	−
	60	2.0	−	2.75
Sacrificed with symptoms	22	3.0	2.75	3.5
	26	<1.0	−	<1.0
	26	2.5	3.3	−‡
Sacrificed healthy	13	1.5	−	−
	20	1.5	−	3.5
	22	1.5	−	2.5
	22	1.5	3.0	3.5
	25	1.5	−	3.0
	26	1.5	−	−
	32	2.5	−	−
	32	+	−	−
	41§	2.5	3.0	3.4
	75	1.5	−	−
	75	1.5	−	−

−, virus not demonstrated.

+, virus present in this tissue; LD_{50} titer not determined.

* Virus inoculum approximately 8,000 mouse intracerebral LD_{50}. Animals which died during the 1st week after virus inoculation are not included in the analysis.

‡ Failure to demonstrate virus may have been due to autosterilization.

§ Animal inoculated suprascapularly.

only. This occurred in one animal found dead on the 24th day after inoculation, in another found dead on the 59th day, and in others sacrificed without evident signs of rabies as long as 2½ months after virus inoculation. Whether or not animals developed symptoms of rabies and recovered could not be clearly established in these experiments because of the difficulty involved in handling these animals. The data indicates, however, that virus may persist in experi-

mentally infected animals for long periods of time and that the brown adipose tissue may serve as a depot for storage of such virus. A small percentage of the bats used in these experiments may have been naturally infected with rabies virus. It is unlikely that this significantly influenced the results of these experiments. The relative insusceptibility of the Mexican free-tailed bat to the massive virus dose used may have been due to the presence of neutralizing antibodies in some of these animals (34).

Since the virus inoculum was the same in each of the three experiments with the Mexican free-tailed bat, the combined experiences were analyzed to determine the frequency with which virus was detectable in brown fat as compared with the other tissues tested. It is significant that in this relatively resistant host, following experimental inoculation, the rabies virus was demonstrated in the brown fat in 23 or 22.1 per cent of the 104 experimentally infected animals as compared with 37 (35.6 per cent) in salivary gland and 91 (87.5 per cent) in the brain. It should be emphasized that rabies virus was demonstrated in the brown adipose tissue almost as frequently as in the salivary gland.

Experiments with the Little Brown Myotis (Myotis lucifugus)

It seemed likely that the little brown myotis might serve as a better host for studying the role of brown adipose tissue in the pathogenesis of rabies because of certain characteristics which differentiate this species from *Tadarida mexicana*. The interscapular brown adipose tissue of *Myotis lucifugus*, a deep hibernator, is a well defined organ-like structure and is more abundant in this species than in *Tadarida mexicana*. Furthermore, the demonstration that certain species of Vespertilionidae have less rabies neutralizing antibodies than do certain species of Molossidae (34) suggests that the little brown myotis may be more susceptible to experimental infection than the Mexican free-tailed bat. The results of experiments with this bat species are presented in Table III.

In one experiment each of 116 bats received an intramuscular injection of approximately 8,000 mouse i.c. LD_{50} contained in 0.1 ml. Those which survived the 1st week of the observation period (97 animals) are considered in the tabulation. Again all animals were observed frequently for signs and symptoms of rabies. Qualitative assays for virus showed evidence of rabies infection during the observation period of 2 months in 35 or 36.1 per cent of the inoculated bats. Among the animals shown to be infected, virus was detectable in the interscapular brown adipose tissue in 9 (25.7 per cent), salivary gland in 4 (11.4 per cent), and in the brain in 32 (91.4 per cent). Similar results were obtained in a smaller group of little brown bats which received the same virus inoculum (8,000 mouse i.c. LD_{50}) into the surgically exposed interscapular brown fat lobes. Rabies infection occurred in 24 or 43.6 per cent of the 46 bats which survived the 1st week of the observation period of 2½ months; virus was demonstrated in the brown fat in 9 (37.6 per cent), salivary gland in 6 (25.0 per cent), and in the brain in 23 (95.9 per cent).

It is clear that the little brown myotis is more susceptible than the Mexican free-tailed bat to the virus strain used; the infection rates were significantly higher and the incubation periods noticeably shorter.

Table IV summarizes the results of virus titrations on tissues from 18 bats in the two previous experiments in which the brown fat contained detectable or significant amounts of rabies virus. The virus is widely distributed in this host between the

TABLE III

Demonstration of Rabies Virus in Interscapular Brown Adipose Tissue and Other Tissues of Experimentally Infected Bats (Myotis lucifugus)

Route of inoculation	No. infected / No. inoculated*	Per cent infected	Time after inoculation‡			Rabies virus demonstrated in§		
			Dead <4 hrs.	Sacrificed with symptoms	Sacrificed healthy	Brown fat	Salivary gland	Brain
			days	*days*	*days*			
i.m.	35/97	36.1	16	19		+	+	+
			13, 20	28		+	−	−
			9, 14, 19	16		+ [25.7]	− [11.4]	+ [91.4]
			13		20	−	+	+
			9–29 (18)	13, 13, 17, 17	14,21	−	−	+
b.f.	24/46	43.6	14, 17	14		+	+	+
			12, 15, 20	26	22	+	−	+
				36		+ [37.6]	− [25.0]	− [95.9]
			18, 18, 22			−	+	+
			16–22 (8)		22 (4)	−	−	+

* Virus inoculum approximately 8,000 mouse intracerebral LD_{50}. Animals which died during the 1st week after virus inoculation are not included in the analysis.

‡ Figure in parentheses refers to number of bats.

§ Figure in brackets indicates per cent positive among animals shown to be infected.

9th and 26th day following i.m. or b.f. inoculation and most active viral multiplication seems to occur during this period. The higher virus titers observed in the tissues assayed reflects the increased susceptibility of the little brown bat as compared with the Mexican free-tailed bats used in the previous experiments. Virus concentration in the brain and in one instance in brown fat approached the level of the stock mouse brain virus suspension used in inoculating these bats. Virus titers in the brain of animals inoculated i.m. or b.f. were generally greater than $10^{-3.0}$, while titers in the salivary gland ranged from $10^{-1.5}$ to $10^{-3.5}$, and in the interscapular brown fat from $10^{-1.5}$ to $10^{-4.3}$. Negri bodies were demonstrated in the brain of one bat which was paralyzed when sacrificed 26 days after b.f. inoculation. The virus titer in the brain was $10^{-4.8}$ and $10^{-4.3}$ in the brown fat. No virus was detected in the salivary gland

TABLE IV

Levels of Rabies Virus in Interscapular Brown Adipose Tissue, Brain, and Salivary Gland of Experimentally Inoculated Bats (Myotis lucifugus)

Route of inoculation	Condition of bats	Time after inoculation*	−Log LD_{50} in mice (i.c.)		
			Brown fat	Salivary gland	Brain
		days			
i.m.	Dead < 4 hours	9	1.5	—	3.5
		13	2.0	—	—
		14	1.5	—	3.0
		16	2.5	3.5	3.7
		19	1.5	1.5	1.5
		20	2.0	—	—
	Sacrificed with symptoms	16	2.5	—	3.5
		19	3.5	1.5	3.7
		28	1.5	—	−‡
	Dead < 4 hours	12	3.5	—	3.7
		14	3.5	3.5	3.7
		15	+	—	3.7
		17	1.5	2.5	4.0
		20	1.5	—	2.0
b.f.	Sacrificed with symptoms	14	2.5	1.5	3.0
		26§	4.3	—	4.8
		36	1.5	—	−‡
	Sacrificed healthy	22	2.5	—	3.5

−, virus not demonstrated.

+, virus present in this tissue; LD_{50} titer not determined.

* Virus inoculum approximately 8,000 mouse intracerebral LD_{50}. Animals which died during the 1st week after virus inoculation are not included in the analysis.

§ Observed with symptoms for 2 days; paralyzed when sacrificed, Negri bodies demonstrated.

‡ Failure to demonstrate virus may have been due to autosterilization.

of this animal. Occasionally virus was demonstrated in the brown fat only; in each instance the titer was $10^{-2.0}$ or less. Low titer virus was demonstrated in the brown fat only of two bats showing overt signs of disease when sacrificed; one 28 days following i.m. inoculation and the other 36 days following b.f. inoculation. Failure to demonstrate virus in the brain of each of these animals may have been due to autosterilization. At no time was virus found in the brown fat and salivary gland but not in the brain in the experiments with the little brown myotis.

It is especially significant that virus was recoverable more frequently from the brown adipose tissue of the experimentally infected little brown bats than

from the salivary glands of these animals; virus was demonstrated in the brown fat of 18 or 30.5 per cent of the 59 experimentally infected animals as compared with 10 or 17.0 per cent in which virus was demonstrated in the salivary gland. Virus was demonstrated in the brain tissue of over 90 per cent of these animals. These data further emphasize the role of the brown adipose tissue in the pathogenesis of rabies and would indicate that this tissue may be as frequent a site of viral proliferation as the salivary gland.

Because of the difficulties inherent in working with winged animals which cannot be colonized in the laboratory it seemed desirable to use a laboratory animal which might provide a more effective means for assessing the role of brown adipose tissue in the pathogenesis of rabies. The Syrian hamster was selected because of the ease with which this animal can be handled and maintained in the laboratory. The young hamster provides a host in which the intramuscular route of inoculation of rabies virus produces the disease almost as regularly as the intracerebral route (35). These animals are significantly more susceptible to experimental infection with rabies virus than the two species of bats used in the previous experiments. In addition, the Syrian hamster which may hibernate when exposed to the cold possesses clearly defined brown fat tissue in the interscapular fossa. The results of two experiments designed to demonstrate rabies virus in interscapular brown adipose tissue and other tissues of experimentally infected hamsters are presented in Table V.

In one experiment each of 42 6-week-old hamsters received an i.m. injection of approximately 2,000 mouse i.c. LD_{50} contained in 0.1 ml. In a second experiment each of nine suckling hamsters received approximately 800 mouse i.c. LD_{50} by the same route. Most of these animals showed signs of rabies infection during the 2nd week after virus inoculation; evidence of infection was demonstrated in 83 per cent of the 6-week-old hamsters and in all of the suckling hamsters. In addition to brown fat, salivary gland, and brain, kidney and a biopsy of muscle at the site of inoculation were tested for rabies virus.

Among the 6-week-old hamsters shown to be infected virus was detectable in the interscapular brown adipose tissue in 22 (62.9 per cent), salivary gland in 8 (22.9 per cent), in the biopsied muscle in 4 or 12.5 per cent of the 32 specimens tested, and in none of the kidneys. The brain tissue from all but three of these animals contained virus. One of these animals was sacrificed on the 9th day after virus inoculation without symptoms of rabies and virus was detected only in the brown fat tissue. The other two animals were found dead on the 9th and 14th day, respectively, and again virus was demonstrated only in the brown fat tissue. Failure to demonstrate virus in the brain or other tissues of these animals may have been due to autolysis or autosterilization. All the suckling hamsters showed signs and symptoms of rabies between the 5th and 12th day after virus inoculation. Rabies virus was demonstrated in at least one of the tissues of each of these animals. The brown fat tissue from an animal which developed symptoms of rabies on the 5th day following virus inoculation was not tested because of insufficient quantity. Of the remaining 8 animals 5 (62.5 per cent) contained virus in the brown adipose tissue. Only one of the five animals had detectable

virus in the salivary gland. This tissue was not tested in the remaining four animals. Virus was demonstrated in the brain tissue of all of the animals and in the kidneys of 3 (33.3 per cent).

Virus concentrations in the interscapular brown adipose tissue and other tissues of experimentally infected hamsters are summarized in Table VI.

TABLE V

Demonstraton of Rabies Virus in Interscapular Brown Adipose Tissue and Other Tissues of the Syrian Hamster Inoculated Intramuscularly

No. infected / No. inoculated	Per cent infected	Time after inoculation			Rabies virus demonstrated in*				
		Dead <4 hrs.	Sacrificed with symptoms	Sacrificed healthy	Brown fat	Salivary gland	Brain	Muscle	Kidney
		days	*days*	*days*					
			10		+	+	+	+	−
		9, 10	8, 9, 10		+	+	+	−	−
			8		+	+	+	NT	−
		9, 14	9-22 (9)		+	−	+	−	−
35/42‡	83.3	16			+ [62.9]	− [22.9]	+ [91.4]	NT	NT
		9, 14		9	+	−	−	−	−
			9, 11, 13,		−	−	+	+	−
			13		−	+	+	−	−
		10	8–17 (5)	5, 8	−	−	+	−	−
		16			−	−	+	NT	NT
			12		+	+	+		+
			8, 15		+	−	+		+
9/9§	100.0		8, 14		+ [62.5]	−	+ [100.0]		− [33.3]
			5, 7, 8		−	NT	+		−
			5		NT	NT	+		−

NT tissue not tested.

* Figure in brackets indicates per cent positive among animals shown to be infected.

‡ Six week old hamsters; virus inoculum approximately 2,000 mouse i.c. LD_{50}.

§ Suckling hamsters; virus inoculum approximately 800 mouse i.c. LD_{50}.

Included in the tabulation are those animals in which the brown fat was shown to contain rabies virus and three additional animals in which virus was not demonstrable in the brown fat but in which significant virus titers were demonstrated in brain and in muscle at the site of inoculation. Virus titers comparable to those observed in white Swiss mice succumbing following intracerebral inoculation were observed in these animals following an intramuscular inoculation; titers ranged from $10^{-3.5}$ to $10^{-5.0}$. Virus was demonstrated in the brain tissue of all of the animals which were sacrificed when symptoms were observed. In many instances the virus titer in the brown fat approached that observed in the brain indicating that viral proliferation had occurred in this tissue. However, there was little evidence of viral multiplication in the brown fat tissue of the suckling hamsters. This may have been due to the fact that this tissue is not fully developed in the immature hamster. In general, the virus concentra-

TABLE VI

Levels of Rabies Virus in Interscapular Brown Adipose Tissue and Other Tissues of the Syrian Hamster Inoculated Intramuscularly

Condition of hamsters	Time after inoculation*	−Log LD_{50} in mice (i.c.)				
		Brown fat	Salivary gland	Brain	Muscle	Kidney
	days					
Dead < 4 hours	9	2.7	2.5	4.8	—	—
	9	3.7	—	5.0	—	—
	9	3.8	—	—	—	—
	10	3.6	4.0	4.3	—	—
	14	3.0	—	4.6	—	—
	14	2.8	—	—	—	—
	16	2.7	—	4.8	NT	NT
Sacrificed with symptoms	8	<1.0	<1.0	4.5	—	—
	8	3.0	2.8	5.0	NT	—
	8‡	1.0	—	4.8	—	—
	9	3.5	2.6	4.8	—	—
	9	2.5	—	4.8	—	—
	9	3.2	—	5.0	—	—
	9	—	—	4.8	2.5	—
	10	4.3	3.2	4.8	3.0	—
	10	1.5	<1.0	3.5	—	—
	10	2.7	—	4.6	—	—
	10	4.0	—	5.0	—	—
	10	3.0	—	4.8	—	—
	11	2.5	—	4.3	—	—
	11	—	—	4.8	4.0	—
	13	—	—	4.3	3.0	—
	12‡	2.0	2.3	5.0	—	2.3
	14‡	<1.0	—	4.3	—	—
	15‡	1.5	—	3.8	—	1.5
	16	3.0	—	4.6	—	—
	22	3.0	—	4.6	—	—
	8‡	<1.0	—	4.8	—	2.0
Sacrificed healthy	9	3.2	—	—	—	—

NT, tissue not tested.

* Six week old hamsters; virus inoculum approximately 2,000 mouse i.c. LD_{50}.

‡ Suckling hamsters; virus inoculum approximately 800 mouse i.c. LD_{50}.

tion in the salivary gland was significantly lower than that observed in other tissues, although a titer of 10^{-4} was observed in one animal which died 10 days after inoculation. Of the three animals which showed virus in the brown fat tissue only one which was sacrificed on the 9th day after inoculation without overt signs of rabies had a virus titer in this tissue of $10^{-3.2}$. The titer in the brown fat of the animal found dead on

the 9th day was $10^{-3.8}$ and a titer of $10^{-2.8}$ was observed in the brown fat of an animal which died 14 days after inoculation. Virus multiplication actually occurred in the muscle at the site of inoculation in some of these animals as evidenced by the fact that virus titers in this tissue ranged from $10^{-2.5}$ to as high as $10^{-4.0}$. In three instances virus was demonstrated in the muscle and brain tissue only and in another virus in high concentration was demonstrated in all the tissues tested except kidney. In the three suckling hamsters in which virus was demonstrated in the kidneys the titers were $10^{-2.3}$ or less. A more detailed sequential study dealing with these and other tissues as sites of viral proliferation is in progress and will be reported subsequently.

DISCUSSION

A viral agent capable of invading host tissue, proliferating without invariably causing overt signs of illness and subsequently transferring to other susceptible hosts, is ideally equipped for continued survival. Epidemiological studies have shown that poliovirus is perpetuated in nature by individuals who have subclinical infections of sufficient intensity to shed infective virus. Severe manifestations of the disease in man are the exception rather than the rule. With other viral agents host-parasite relationships are not always so well balanced and biological life cycles may require multiple hosts. The arbor viruses, for example, may establish infection chains involving one or more vertebrate hosts and various insect vectors. While much study has been done on the epidemiology of the arbor viruses little is known about the true mechanism by which these agents survive between epidemics. Rabies presents still another epidemiological picture. There is little evidence that the principal hosts (domestic dogs and wild carnivores) may serve as symptomless carriers of the virus and hence provide a means for maintaining the agent in nature. The epidemiology of rabies has been the subject of much investigation yet precise mechanisms involved in the maintenance of the virus in widely scattered geographic areas are not clearly understood. The first recognition of rabies in cattle in South America resulting from exposure to infected vampire bats pointed to a previously unknown host in which the virus might be perpetuated *via* the carrier state. The observations of Pawan (4, 5) and others demonstrate clearly that various species of bats may develop overt signs and symptoms of rabic infection and recover, remaining carriers for indeterminant periods of time. In this connection, naturally infected vampire bats capable of transmitting the disease have been held in captivity for several months and remained apparently healthy. Since these early observations, the disease in these animals seems to have moved northward causing outbreaks in animals and man in many areas of South and Central America and Mexico (6).

Demonstration of rabies virus in several species of apparently healthy colonial and non-colonial insectivorous species of bats collected in the United States in recent years suggests that these animals may also be reservoirs for storage of the virus in nature. The regimented flight habits of certain migrating

insectivorous bats bring them in contact with vampires in Mexico where the various species may live together in caves, providing opportunity for interspecies spread of virus. In addition, certain physiological characteristics common to bats suggest an approach to the study of the mechanism by which these animals may store virus for long periods of time without showing overt signs of infection. In certain species of bats, and other hibernating animals, interscapular brown adipose tissue is a well developed, organized structure and for this reason is often, perhaps erroneously, called the "hibernating gland." It is now well established that this structure which is histogenetically distinct from ordinary white adipose tissue and physiologically more active (36) is not a mere storage form of connective tissue but shows high metabolic activity, storing glycogen in concentrations comparable with that in the liver (37, 38). Brown adipose tissue is a rich source of certain lipides which are of prime importance in the maintenance of the hibernating animal and are not simply static reserves but fluctuate with the seasonal variations in the physiology of hibernating animals. The total lipide content of brown adipose tissue obtained from *Myotis lucifugus* reaches a peak during the month prior to entering hibernation, gradually decreases during the period of inactivation and increases again in preparation for the awakening. This variation in level of total lipides may be an indication of the general metabolic state of these bats (39). In addition to lipides, brown adipose cells of various animal species have been shown to contain ascorbic acid, alpha amino acids, mucoproteins, water-soluble polysaccharides, glycogen, amine oxidase, cytochrome oxidase, alkaline phosphatase, succinic dehydrogenase, and esterase (23, 36, 39, 40). Brown adipose tissue has been found to be highly vascularized, capable of supporting considerable metabolic activity and to have an abundant nerve supply which seems to influence the storage and utilization of glycogen and lipides (36, 39, 41, 42). Interscapular brown fat of fetal and postnatal rats has been grown in organ culture and although tissue survived for only a few days, all stages in the differentiation of brown adipose tissue were observed *in vitro* (43). Further evidence that brown fat is an actively metabolizing tissue has been obtained in our laboratory by the serial propagation of trypsinized hamster and bat brown adipose tissue. Original cultures have been maintained for as long as 30 days and the cells have been carried successfully through five serial passages *in vitro*. Preliminary studies indicate that cell cultures of hamster brown fat tissue support the growth of rabies virus without significant cytopathogenic changes. Infection of these cultures with herpes virus (strain HF-egg adapted) results in dramatic cellular degeneration which can be neutralized by specific immune serum (44). These observations suggest that brown adipose tissue would provide an ideal substrate for maintenance or replication of the rabies virus. The rate of proliferation of the virus might conceivably fluctuate with the variation in metabolic activity of this tissue, decreased to the point of latency during

periods of low metabolism (hibernation) and becoming more active when metabolism increases in preparation for awakening.

Studies presented in this report clearly indicate that brown adipose tissue may serve as a depot for storage and an extraneural site of multiplication of rabies virus. Rabies virus was isolated from the brown fat tissue of experimentally infected animals with frequencies ranging from 22 per cent in the relatively insusceptible Mexican free-tailed bat to almost 62 per cent in the highly susceptible Syrian hamster. Quantitative assays of infected brown fat tissues revealed titers equal to the mouse i.c. LD_{50} titer of the rabies virus inoculum indicating active multiplication of the virus. A comparison of results of the qualitative and quantitative viral assays of the brown fat and salivary gland of infected animals reveals that brown adipose tissue provides a site for virus multiplication as frequently as salivary gland. Whether or not rabies virus can be demonstrated in the brown adipose tissue of naturally infected animals remains to be determined. Studies along this line are in progress. Investigation into the morphological relationship of the interscapular brown fat and parotid glands in the species of bats used in these experiments was prompted by the observation of Wimsatt (45) that a large lobe of the parotid gland in the tropical fruit bat, *Artibeus jamaicensis*, extends into the interscapular space to be intimately associated with the brown fat. Studies reported elsewhere (46) indicate that the homogeneity of each of these tissues together with the encapsulation of the interscapular brown adipose tissue by a loose fascial covering in the bat species, *Tadarida mexicana* and *Myotis lucifugus*, precludes the possibility of "contamination" of one with the other.

It seems to be clearly established that the salivary gland becomes infected with rabies virus by centrifugal spread and overflow from the central nervous system. The exact means by which the central nervous system becomes infected is not known although a neural pathway seems likely. The studies here reported would suggest that virus may reach the brown fat by direct extension from the site of inoculation. Unfortunately the data contained in this report do not allow analysis of the exact sequence by which the virus spreads in the very early period following inoculation. Whether or not virus reaches this tissue by a hemal route remains to be determined. A limited number of experiments in the Syrian hamster suggest that this may be the case. In animals in which the interscapular brown fat lobes were denervated virus could be demonstrated in this tissue following intramuscular inoculation at a remote site. Further details will be published subsequently.

Precise mechanisms by which brown fat may actually serve as a depot for storage of rabies virus is obviously not yet clearly understood. Just how virus is released from this tissue to participate in the natural history of the disease remains to be determined. It is conceivable that at least in hibernating species both the virus and the host remain quiescent during the period of hibernation,

the virus being activated by those physiological alterations (stresses) which prepare the animal for the awakened period. To establish whether or not this may be the case, studies concerned with the influence of environmental temperature and the gravid state on the pathogenesis of rabies in insectivorous bats have been initiated and the results will be reported subsequently.

Similar mechanisms may be involved in the maintenance of other viral agents during interepidemic periods. The observation that Western equine encephalomyelitis virus may infect garter snakes (47) would suggest that in this cold blooded animal there may be a tissue similar to the "hibernating gland" of warm blooded animals which may serve as a storage site for virus during the winter months. A mosquito-bat-mosquito cycle has been recently suggested for Japanese encephalitis and Venezuelan equine encephalomyelitis viruses on the basis of studies on experimentally infected bats during simulated hibernation (48, 49). Preliminary experiments in this laboratory have already indicated that the brown adipose tissue in the bat may be involved in the pathogenesis of certain arbor viruses and the mechanism for storage may be similar to that suggested for the rabies virus (50).

SUMMARY

Studies on the pathogenesis of rabies in two species of experimentally infected insectivorous Chiroptera, the Mexican free-tailed bat (*Tadarida mexicana*), a quasi hibernator, and the little brown bat (*Myotis lucifugus*), a deep hibernator, provided evidence that brown adipose tissue may serve as an extraneural site for storage and multiplication of rabies virus. Although the Mexican free-tailed bat proved to be relatively insusceptible to experimental rabies infection, virus was demonstrated in the brown fat of 22 per cent of those animals shown to be infected by viral assay in white Swiss mice. Rabies infection in this species was most evident 20 to 40 days after intramuscular inoculation of virus. Rabies virus was found to be widely distributed in the little brown myotis 9 to 26 days following inoculation and virus concentrations in some of the tissues approached the level of the stock mouse brain virus suspension used in inoculating these bats. The shorter incubation period and higher virus titers in the tissues assayed reflect the increased susceptibility of *Myotis lucifugus* as compared with the Mexican free-tailed bat. Virus was demonstrated in the brown fat of 30 per cent of the experimentally infected *Myotis*.

In the experimentally infected *Myotis lucifugus* and in the Syrian hamster which is highly susceptible to rabies infection, rabies virus was isolated more frequently from the brown fat than from the salivary gland indicating that in a susceptible host brown adipose tissue may be as frequent a site of viral proliferation as salivary gland.

Since rabies virus was found to persist for long periods of time in the brown fat of experimentally infected bats and was occasionally demonstrated in this

tissue alone, it is suggested that brown adipose tissue provides a mechanism by which these animals may serve as reservoirs for this agent in nature. The possibility that similar mechanisms may be involved in the maintenance of other viral agents during interepidemic periods is discussed.

The authors wish to acknowledge the excellent technical assistance of H. Warrington Williams and Chansoo Kim, M.D.

BIBLIOGRAPHY

1. Johnson, H. N., *in* Viral and Rickettsial Infections of Man, (Thomas M. Rivers and Frank L. Horsfall, Jr.), editors, Philadelphia, J. B. Lippincott Co., 3d edition, 1959, 405.
2. Koprowski, H., Latent and dormant viral infections, *Ann. New York Acad. Sc.*, 1952, **54**, 963.
3. Lima, E. de Queiroz, A transmissão da raiva dos herbívoros pelos morcegos hematophagos da familia Desmodontidae, *Rev. Dept. Nac. Prod. Animal*, 1934, **1**, 165.
4. Pawan, J. L., Rabies in the vampire bat of Trinidad, with special reference to the clinical course and latency of infection, *Ann. Trop. Med. and Parasitol.*, 1936, **30**, 401.
5. Pawan, J. L., Fruit-eating bats and paralytic rabies in Trinidad, *Ann. Trop. Med. and Parasitol.*, 1948, **42**, 173.
6. Enright, J. B., Bats and their relation to rabies, *Ann. Rev. Microbiol.*, 1956, **10**, 369.
7. Venters, H. D., Hoffert, W. R., Scatterday, J. E., and Hardy, A. V., Rabies in bats in Florida, *Am. J. Pub. Health*, 1954, **44**, 182.
8. Sullivan, T. D., Grimes, J. E., Eads, R. B., Menzies, G. C., and Irons, J. V., Recovery of rabies virus from colonial bats in Texas, *Pub. Health Rep.*, 1952, **69**, 766.
9. Burns, K. F., and Farinacci, C. J., Rabies in nonsanguivorous bats in Texas, *J. Infect. Dis.*, 1955, **97**, 211.
10. Steele, J. H., personal communication.
11. Tierkel, E. S., Recent developments in the epidemiology of rabies, *Ann. New York Acad. Med. Sc.*, 1958, **70**, 445.
12. Nikolic, M., and Jelesić, Z., Isolation of rabies virus from insectivorous bats in Yugoslavia, *Bull. World Health Org.*, 1956, **14**, 801.
13. Scatterday, J. E., Bat rabies in Florida, *J. Am. Vet. Med. Assn.*, 1954, **124**, 125.
14. Witte, E. J., Bat rabies in Pennsylvania, *Am. J. Publ. Health*, 1954, **44**, 186.
15. Irons, J. V., Eads, R. B., Grimes, J. E., and Conklin, A., The public health importance of bats, *Texas Rep. Biol. and Med.*, 1957, **15**, 292.
16. Sulkin, S. E., and Greve, M. J., Human rabies caused by bat bite, *Texas State J. Med.*, 1954, **50**, 620.
17. Lennette, E. H., Soave, O. A., Nakamura, K., and Kellog, G. H., A fatal human case of rabies following the bite of a rabid bat (*Lasionycteris noctivagans*). Isolation and identification of the virus from vector and victim, *J. Lab. and Clin. Med.*, in press.

18. Shwartzman, G., Enhancing effect of cortisone upon poliomyelitis infection (strain MEF1) in hamsters and mice, *Proc. Soc. Exp. Biol. and Med.*, 1950, **75,** 835.
19. Shwartzman, G., Poliomyelitis infection in cortisone-treated hamsters induced by the intraperitoneal route, *Proc. Soc. Exp. Biol. and Med.*, 1952, **79,** 573.
20. Pappenheimer, A. M., Daniels, J. B., Cheever, F. S., and Weller, T. H., Lesions caused in suckling mice by certain viruses isolated from cases of so-called non-paralytic poliomyelitis and of pleurodynia, *J. Exp. Med.*, 1950, **92,** 169.
21. Dalldorf, G., The Coxsackie viruses, *Bull. New York Acad. Med.*, 1950, **26,** 329.
22. Godman, G. C., Bunting, H., and Melnick, J. L., The histopathology of Coxsackie virus infection in mice. I. Morphologic observations with four different viral types, *Am. J. Path.* 1952, **28,** 223.
23. Aronson, S. M., and Shwartzman, G., The histopathology of brown fat in experimental poliomyelitis, *Am. J. Path.*, 1956, **32,** 315.
24. Bodian, D., Poliovirus in chimpanzee tissues after virus feeding, *Am. J. Hyg.*, 1956, **64,** 181.
25. Sulkin, S. E., Krutzsch, P., Wallis, C., and Allen, R., Role of brown fat in pathogenesis of rabies in insectivorous bats (*Tadarida b. mexicana*), *Proc. Soc. Exp. Biol. and Med.*, 1957, **96,** 461.
26. Krutzsch, P. H., and Sulkin, S. E., The laboratory care of the Mexican free-tailed bat, *J. Mamm.*, 1958, **39,** 262.
27. Burns, K. F., Farinacci, C. F., and Murnane, T. G., Insectivorous bats naturally infected with rabies in the southwestern United States, *Am. J. Pub. Health,* 1956, **46,** 1089.
28. Stamm, D. D., Kissling, R. E., and Eidson, M. E., Experimental rabies infection in insectivorous bats, *J. Infect. Dis.*, 1956, **98,** 10.
29. Reed, L. J., and Muench, H., A simple method of estimating fifty per cent endpoints, *Am. J. Hyg.*, 1938, **27,** 493.
30. Twente, J. W., Jr., Ecological observations on a colony of *Tadarida mexicana*, *J. Mamm.*, 1956, **37,** 42.
31. Burns, K. F., and Farinacci, C. J., Virus of bats antigenically related to St. Louis encephalitis, *Science,* 1956, **123,** 227.
32. Sulkin, S. E., Wallis, C. and Allen, R., Relationship of bat salivary gland virus to St. Louis encephalitis group of viruses, *Proc. Soc. Exp. Biol. and Med.*, 1956, **93,** 79.
33. Levaditi, C., Sanchis-Bayarri, V., and Schoen, R., Neuro-infections autosterilisables (encephalite, herpis, rage), *Compt. rend. Soc. biol.*, 1928, **98,** 911.
34. Burns, K. F., Shelton, D. F., and Grogan, E. W., Bat rabies: experimental host transmission studies, *Ann. New York Acad. Sc.*, 1958, **70,** 452.
35. Koprowski, H., Experimental studies of rabies virus, *Canad. J. Pub. Health,* 1949, **40,** 60.
36. Fawcett, D. W., A comparison of the histological organization and cytochemical reactions of brown and white adipose tissues, *J. Morphol.*, 1952, **90,** 363.
37. Tuerkischer, E., and Wertheimer, E., Glycogen and adipose tissue, *J. Physiol.* 1942, **100,** 385.

38. Wertheimer, E., and Shapiro, B., The physiology of adipose tissue, *Physiol. Rev.*, 1948, **20,** 451.
39. Remillard, G., Histochemical and microchemical observations on the lipids of the interscapular brown fat of the female vespertilionid bat *Myotis lucifugus lucifugus*, *Ann. New York Acad. Sc.*, 1958, **72,** 1.
40. Menschik, Z., Histochemical comparison of brown and white adipose tissue in guinea pigs, *Anat. Rec.*, 1953, **116,** 439.
41. Rasmussen, A. T., The so-called hibernating gland, *J. Morphol.*, 1924, **38,** 147.
42. Sidman, R. L., and Fawcett, D. W., The effect of peripheral nerve section on some metabolic responses of brown adipose tissue in mice, *Anat. Rec.*, 1954, **118,** 487.
43. Sidman, R. L., Histogenesis of brown adipose tissue *in vivo* and in organ culture, *Anat. Rec.*, 1956, **124,** 581.
44. Sulkin, S. E., Wallis, C., Allen, R., and Sims, R. A., Monolayer cultures of trypsinized bat and hamster brown adipose tissue. Response to virus infection, data to be published.
45. Wimsatt, W. A., On the nature of the interscapular gland of the tropical American fruit bat *Artibeus jamaicensis* Leach, *Anat. Rec.*, 1955, **121,** 549.
46. Krutzsch, P. H., and Sulkin, S. E., The anatomical distribution of the interscapular and parotid glands of insectivorous bats, *Tadarida*, *Myotis*, and *Pipistrellus*, *Anat. Rec.*, in press.
47. Thomas, L. A., Eklund, C. M. and Rush, W., Susceptibility of garter snakes (*Thamnophis* spp.) to Western equine encephalomyelitis virus, *Proc. Soc. Exp. Biol. and Med.*, 1958, **99,** 698.
48. Corristan, E. C., LaMotte, L. C., Jr., and Smith, D. G., Susceptibility of bats to certain encephalitis viruses, *Fed. Proc.*, 1956, **15,** 584.
49. LaMotte, L. C., Jr., Japanese B encephalitis in bats during simulated hibernation, *Am. J. Hyg.* 1958, **67,** 101.
50. Sulkin, S. E., Allen, R., and Sims, R. A., Insectivorous bats in the epidemiology of encephalitis: A suggested mechanism, data to be published.