is the last step in biosynthesis of norepinephrine. The finding that 3-hydroxytyramine is a precursor of norepinephrine is in agreement with many studies *in vitro*. However, these findings are the first evidence of synthesis of norepinephrine from 3-hydroxytyramine *in vivo*. It is known that certain stresses increase excretion of norepinephrine(8), and in our stress experiments a higher excretion of 3-methoxynorepinephrine was also observed.

Of special interest may be the discrepancy in the biological half-life of 3-hydroxytyramine and norepinephrine. It was found that labeled 3-hydroxytyramine was no longer excreted 48 hours after last injection of 3-hydroxytyramine-1-C^{14}. However, radioactive 3-methoxynorepinephrine was still being excreted 8 days after last injection of 3-hydroxytyramine. Although a more quantitative study is necessary to permit exact calculation of turnover rates of these 2 catecholamines, it is evident that their half-life is different.

The fact that norepinephrine-C^{14} is stored for at least 8 days, while 3-hydroxytyramine-1-C^{14} appears to be completely replaced by synthesis of endogenous hydroxytyramine in 48 hours, suggests that the turnover rate of dihydroxyphenylalanine to hydroxytyramine is faster than the turnover of 3-hydroxytyramine to norepinephrine. This may mean that conversion of hydroxytyramine to norepinephrine is the rate-limiting step in the synthesis.

Summary. 1. Formation *in vivo* of norepinephrine from 3-hydroxytyramine-1-C^{14}, can be demonstrated indirectly by isolating the end product of biosynthesis, namely, 3-methoxynorepinephrine. 2. Labeled 3-methoxynorepinephrine was isolated as a diacetate derivative from urine of unstressed and stressed rats treated with iproniazid and 3-hydroxytyramine-1-C^{14}. Radiochemical purity of the diacetate and the *N*-acetyl derivative was established by paper chromatography, and by recrystallization of the diacetate derivative to constant specific activity. 3. Radioactivity of 3-methoxynorepinephrine isolated from urine of rats was higher in stressed rats. 4. Labeled 3-methoxynorepinephrine continued to be excreted for 8 days after treatment with 3-hydroxytyramine-1-C^{14} in contrast to radioactive 3-hydroxytyramine and 3-methoxytyramine which disappeared from urine after 48 hours.

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Rabies Virus Isolated from Brown Fat of Naturally Infected Bats. (25438)

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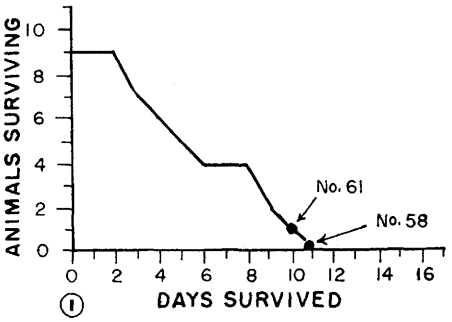
The physiological nature of brown fat is obscure, but is now receiving increased attention primarily because of numerous isolations of several kinds of viruses from brown fat of various animals(1) and an inference that it may play a special role in host-virus relationship(2). Sulkin *et al.*(1) adduced evidence from bats experimentally infected with rabies virus that this virus may be present in brown fat when absent from other organs, and that it may propagate in this tissue. We here present evidence of the presence of rabies

virus in brown fat of naturally infected bats: that is in pooled brown fat and pooled brain of 2 *Myotis lucifugus* and in brown fat, brain, and salivary gland of one *Eptesicus fuscus*.

Materials and methods. Swiss white mice, 21 days old (weanling), of Rocky Mountain Laboratory strain, were used routinely for isolation. Two-day-old mice were also injected in tests repeated when presence of virus in low titer was suspected. Tissues tested were brain, salivary glands, and brown fat. The last 2 tissues are near each other anatomically but grossly discrete and distinct in the 2 genera under consideration and the chance of contamination of one by the other at necropsy is remote. After bats were dead, tissues were ground by mortar and pestle in 10% albumin-saline diluent at pH 7.5 to make suspensions estimated to be 10%, and 0.03 ml were injected into mice by intracerebral route. Excess suspension was preserved in mechanical deep-freezer. Bats of the species *Myotis lucifugus* and *Eptesicus fuscus* were obtained in routine collections from attics of homes in western Montana. They were held overnight in battery jars with water available. The day following collection, they were hand-fed canned dog food* to which about 10% of an homogenate of meal worms, fresh guinea pig liver and vitamins† was added. Immediately following feeding they were banded and released into indoor flight cages (4′4″ x 5′ x 16″). No special provision was made for temperature or humidity control. The usual course of events when *E. fuscus* and *M. lucifugus* are treated thus is that an initial high mortality for several days is followed by slow attrition of survivors over several months. The group of *Eptesicus* from which the infected animals were obtained was not exceptional in this regard but the small group of *Myotis* succumbed rapidly. Signs of rabies were not observed in the infected bats and it cannot be stated that their deaths were caused by rabies. The status of infection in the animals can be inferred only to a limited extent by comparing the time of their deaths with that of other bats in the cages (Figs. 1 and 2).



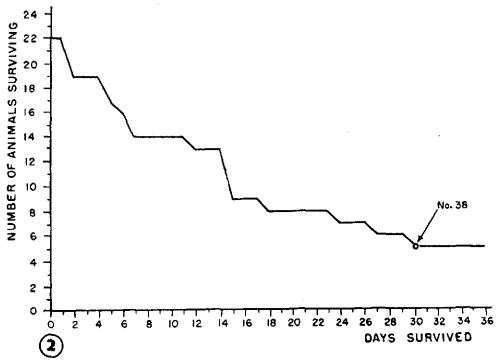


FIG. 1. Mortality in group of *M. lucifugus*.
FIG. 2. Mortality in group of *E. fuscus*.

Results. *Myotis* bats No. 61 and 58 died on 10th and 11th days of captivity, respectively, and were the last of the group to die. Carcasses were kept at 4°C until one day after death of No. 58, when they were necropsied and like tissues were pooled and injected. Rabies virus was isolated from pooled brain and from pooled brown fat after incubation periods of 16 and 18 days respectively. Five of 6 mice became infected from brain tissue and 1 of 6 from brown fat. Typical Negri bodies were found in mouse brains. Pooled salivary glands failed to infect mice on primary inoculation, and the preserved glands also failed to infect suckling mice in a subsequent test.

One hemisphere of each of the *Myotis* brains was fixed in Zenker's fluid and stained with Williams' stain. One atypical Negri body, *i.e.*, without internal structure, was found in the brain of No. 58. No other lesions could be detected.‡

Eptesicus (No. 38) died on 30th day of

*Pard.

†Abdec brand.

‡ Pathology by Dr. L. L. Ashburn, Nat. Inst. Health.

captivity and was necropsied the same day. One of 6 mice injected with brown fat suspension and all mice injected with brain or salivary gland suspensions died of rabies. Incubation periods were 22, 9, and 11 days, respectively. Titers of virus in *Eptesicus* brain and salivary glands were log 1.2 and 3.4/0.03 ml respectively. Negri bodies were not found in mouse brains. Sections of *Eptesicus* brain fixed in formalin were stained with azure-eosin and with hematoxylin and eosin. No pathognomonic changes could be discerned.

The long incubation periods of isolates from brown fat of *Myotis* and *Eptesicus* were shortened to 10 days in the second mouse passage.

Discussion. Our demonstration of rabies virus in brown fat of naturally infected insectivorous bats agrees with the findings of Sulkin *et al.*(1) who worked with experimentally infected bats. Presence of virus in brown fat when absent from salivary glands was also noted by us and by Sulkin, but in our observations the difference may not have been significant, inasmuch as only a minimal amount of virus was found in the brown fat of *Myotis*. It is quite possible that deaths of these animals were unrelated to infection inasmuch as other bats maintained identically also died but without infection. Dubiety regarding infection as cause of death may be indicated also because of absence of encephalitic lesions in the brain.

Rabies virus has been isolated by us from 12 bats of 7 species collected in western Montana. In only 2 of those instances have we attempted isolation from brown fat and both attempts were successful. We also tested tissues of 106 *Myotis, Eptesicus,* and *Corynorrhinus* but have not found virus in brown fat when absent from both brain and salivary glands.

A discussion of the role of brown fat in pathogenesis of rabies may be found in the paper by Sulkin *et al.*(1).

Summary. Rabies virus has been isolated from brown fat of naturally infected *Eptesicus* and *Myotis* bats. In each case virus was present in very low infective concentration. Rabies virus was also isolated from brains of the same bats and from salivary glands of the *Eptesicus*.

Addendum. Rabies virus has since been isolated from brown fat in 5 more instances including 3 from species not mentioned above. Two isolates were from *Lasionycteris noctivagans* and one from *M. evotis*.

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Susceptibility Rhythm to *E. coli* Endotoxin and Bioassay.* (25439)

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In toxicity testing, variations of "response" along a 24-hour time scale are usually not evaluated. Most reviews or books on the subject actually omit discussions of physiologic rhythms in relation to bioassays in general, since available references are sporadic and questionable. The contribution of physiologic rhythms to variability of assays thus awaits rigorous evaluation, the outcome of which will depend largely upon control of sources of variation other than rhythms, such as genetics, past history, physical environment as well as sampling effects. Control of such factors was attempted in this study on the significance and reproducibility of a possible susceptibility rhythm to endotoxin. Can the latter rhythm be unmasked from interference

* Supported by Elsa U. Pardee Fn., Am. Cancer Soc., U.S.P.H.S., and Dept. of Public Welfare, State of Minnesota.