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

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by other factors so that periodic changes in the body's response to a poison stand out in clear view of all? This aim was approximated by light-synchronized periodicity analysis: a dramatic susceptibility rhythm was found. In view of its reproducibility and significance under the stated conditions, its bearing upon bioassay also was experimentally explored.

Materials and methods. C (Bagg albino) mice, brother-to-sister mated for over 10 years in our laboratory, were studied. For at least 7 days prior to each experiment, the mice were singly caged at 24°C, in light daily from 06 to 18, with food and water continuously available. In each of 2 experiments, 7 groups of mature animals matched by age, sex, and weight were injected intraperitoneally at 4-h intervals with 100 μg/.2 cc/20 g body weight of Difco's *E. coli* lipopolysaccharide.† A third experiment consisted of 2 LD_{50} assays done 12 hours apart in mice that were again comparable in terms of genetic background, sex, age, past history, and experimental conditions.

Results. At 1 week post injection, as well as at earlier time-points, differences in mortality among some groups of mice injected at different times were large and significant below 1%; equally important, with the dose and stock used, these differences were reproducible from first to second experiment (Fig. 1), probably because of standardization of experimental conditions.

In the third experiment, a large difference in "potency" was seen in 2 assays made 12 hours apart with samples from the identical batch (Fig. 2); the computed "relative potency" of the same material tested at 12 and 24 was 3.22. This inter-assay difference is clearly a function of stage of susceptibility rhythm in which the injections were made. A comparison of responses at the 2 dose levels common to both assays demonstrated the significance of the difference ($P<.001$). Under our conditions, the stage of rhythm selected for testing was a much more significant source of variation than several other factors tested, such as position of cage on rack, or the type

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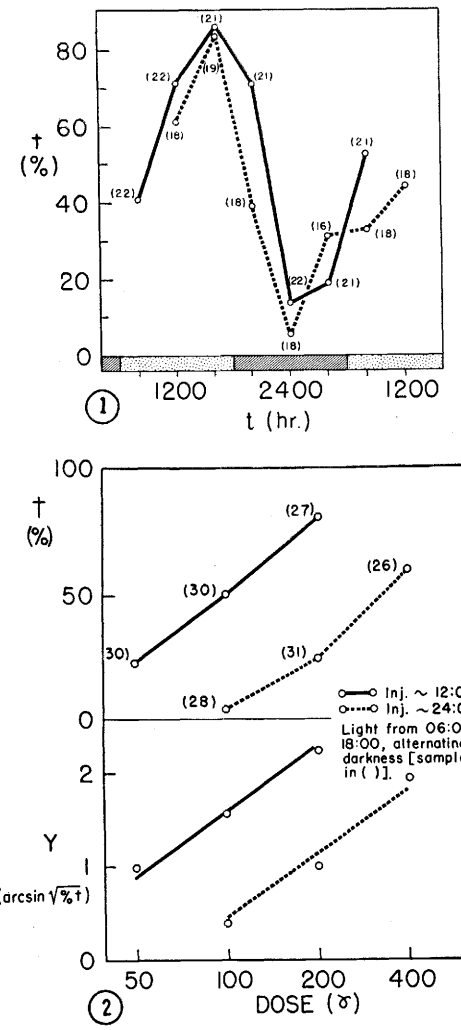

FIG. 1. Susceptibility rhythm to endotoxin and its reproducibility in separate experiments. Ordinate: % death from Difco's *E. coli* lipopolysaccharide (100 μg/20 g, i.p.) in separate groups of *standardized* mature C mice inj. at 4-hr intervals. Abscissa: times of inj. in 2 exp. begun at different times during daily light period. No. of mice/group in parentheses. Evaluation at 1 wk post inj.

FIG. 2. Different "potency" of samples from same endotoxin batch tested at different times, shown by mortality of C mice inj. in different phases of their daily susceptibility rhythm. Evaluation at 18 hr post inj.

(not the schedule!) of illumination tested (results not shown; $P>.10$).

Discussion. Standardization for light-dark synchronized periodicity analysis is desirable for bioassay as well as for other experimental work(1): Variability will be reduced by testing synchronized populations in defined phases

of 24-hour rhythm. In the absence of such standardization intra-assay variability may be augmented by dissociation of susceptibility rhythms among individuals and inter-assay variability by testing in different stages of rhythm.

Control of clock-hour of testing does not necessarily suffice for reducing the contributions of rhythms to variability of bioassays; rhythms might be free-running from the local clock with periods slightly though consistently different from 24 hours(1,2). Standardization for light-synchronized periodicity analysis overcomes this difficulty: "physiologic time" in terms of stage of susceptibility rhythm can then be related to a given clock hour. Subsequent work shows further that timing of peak (or trough) in susceptibility to endotoxin can be shifted from the usual clock hour by appropriate manipulation of the lighting regimen and by observing several additional simple precautions such as avoidance of disturbances in the mouse room, *e.g.*, from presence of experimenters(1). Work carried out in reproducibly defined stages of a given physiologic rhythm is particularly critical when it allows for detection (in one stage of rhythm) of effects which might not otherwise be found (in tests done in another stage of rhythm)(3). Apart from methodologic considerations, susceptibility rhythms show how critically the stage of *periodic* changes in physiologic state can determine an organism's ability to withstand damage.

Summary. Light-synchronized periodicity analysis reveals a susceptibility rhythm in C mice to *E. coli* endotoxin. Susceptibility varies predictably and significantly along the 24-hour time scale. A dose of endotoxin which is compatible with survival of most animals when given during middle of daily dark period is highly lethal when it is given 8-12 hours earlier or later. LD_{50} also was determined for 2 samples from identical batch of endotoxin tested 12 h apart on separate groups of comparable C mice kept under conditions standardized for periodicity analysis: Differences in potency significant at 1% were seen at 2 dose levels common to both assays, the computed "potency ratio" being 3.22.

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Inhibitory Influence of Ethanol on Serotonin Metabolism. (25440)

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During a study of acute experimental intoxication using normal human subjects, it was observed that a pronounced decrease in urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) occurred during 5-hour collection period following ingestion of about 100 g of ethanol. Inasmuch as 5-HIAA is the major metabolic end-product of serotonin, (5-hydroxytryptamine) metabolism in man(1,2), this finding conjured up the possibility that during metabolism of alcohol the oxidative conversion of endogenous serotonin (and, perhaps, of other monoamines) is temporarily inhibited. Further circumstantial evidence in support of this hypothesis was adduced from preliminary pharmacological studies using mice, which demonstrated that doses of serotonin and other primary aromatic amines, *viz.*, tryptamine, dopamine, and tyramine, which *per se* were innocuous and provoked little somatic, autonomic, or behavioral effects, exerted a striking potentiation of the narcotic action and acute toxicity of alcohol. Prompted by the possibility that the neuropharmacologic

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