Table 1. Radioactivity in the blood of sleeping and waking animals, in counts (multiplied by 10^{-5}) per minute per milliliter.

State of animals	Time after injection	
	10 min*	30 min†
Sleeping	5.3 ± 0.4	3.8 ± 0.3
Waking	4.9 ± 0.1	3.7 ± 0.3

* $N = 16$. † $N = 70$; these animals are those from which the data of Fig. 1 were obtained.

blood flow or in the permeability of the blood-brain barrier to phosphate during sleep might be expected to affect the specific activity of inorganic phosphate in the brain and, consequently, the specific activity of more than one of the brain fractions we studied. However, only fraction II exhibited changes due to sleep. To check whether the effect might in some way be due to $^{32}P_i$ in blood pooled in the brain post mortem, we conducted the following experiment. We added a known amount of $^{32}P_i$ to nonradioactive rat brain homogenate in chloroform-methanol (2 : 1) and followed our usual procedures to the hot trichloroacetic acid extract. Less than 0.05 percent of the initial radioactivity remained, an amount that would correspond to less than 0.4 percent of that found in fraction II in the experiments on the effect of sleep. The effect thus indeed appears to be the result of an increase in the incorporation of phosphate by a constituent of brain tissue during sleep, and not a reflection of the availability of $^{32}P_i$.

The component of fraction II in which the sleep effect was found was shown above to be inorganic phosphate. In view of the previous treatment of the tissues and the experiment just cited, this inorganic phosphate was split probably from a more complex substance by the hot acid. We are attempting to characterize the constituent implicated by the application of specific extraction procedures.

Extraction of RNA (see *11*) and of phosphoinositides (*12*) from the brains of S and W animals indicates that these compounds are not involved in the sleep effect. Pronase (*13*) treatment of the residue after chloroform-methanol extraction fails to solubilize a labeled entity with increased activity during sleep. The phosphate group involved is not susceptible to the action of alkaline phosphatase (*14*). Alkaline hydrolysis at *p*H 8 and *p*H 12 for 15 minutes at room temperature fails to release the relevant phosphate. We conducted all these procedures on brains split down the midline and ran parallel extractions by our usual methods with trichloroacetic acid in order to insure that the sleep effect in fraction II was present. Phosphoproteins are the most likely possibility for the fraction in which the effect is manifested.

Many questions are raised by this report. Not only is the parent compound from which the inorganic phosphate is split in fraction II still to be defined, but the relationships of the observed effect to the phases of sleep and the age and species of the experimental animal remain to be investigated. We report the effect of sleep on the metabolism of a phosphate-containing fraction of brain at this stage because it is reproducible and because of the paucity of available information on brain metabolism during sleep.

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Toxicity of Antibiotics in Laboratory Rodents

Killby and Silverman [*Science* **156**, 264 (1967)] were apparently unaware of several studies on the toxicity of certain antibiotics to small animals. D. Hamre, G. Rake, C. McKee, and H. MacPhillamy [*Amer. J. Med. Sci.* **206**, 642 (1943)] described the toxic effect of penicillin in guinea pigs, and G. Rake and I published toxicity data on streptomycin and dihydrostreptomycin in mice [*J. Bacteriol.* **53**, 205 (1947)].

The authors were also apparently unaware of the need to measure therapeutic doses of any drug in terms of the body weight of the recipient. Thus they did not realize that 4 mg/20 g of mouse body weight means 200 mg/kg of body weight, a toxicity which certainly does not fit into the category of "extremely toxic" when measured against the therapeutic dose.

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Donovick is, of course, quite correct in stating that we were apparently unaware of several studies of the toxicity of antibiotics to small animals. It is furthermore the case that many of our colleagues and professional contacts we made in pharmaceutical firms were also unaware of such toxicity. This is exactly the reason why we wrote calling attention to our "ignorance" in the hope that other investigators would be saved the unhappy experience which we had. It appears that persons not directly involved in the area of antibiotic research could be misled by simply following directions as indicated in the package inserts of some of these drugs.

We consider that, rather than wasting space by publishing our note, we have elicited two valuable literature references provided by Donovick.

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