Pyramitone, and covered with autoradiographic stripping film (Kodak AR 10), as described previously[9]. The exposure time was approximately 3 months.

In the autoradiographs (Figure) radioactivity was observed mainly over the inner plexiform layer of the retina with no apparent sublayering. There was also considerable activity over the nerve fibre layer. Certain cells with the position of amacrine cells (in the innermost cells of the inner nuclear layer) also showed marked radioactivity. The remaining parts of the retina showed only a slight and diffusely distributed radioactivity. Compared with the pattern seen in autoradiographs made from retinas from rabbits, rats, and guinea-pigs[9,13] produced under similar experimental conditions, the similarity is striking. In rat and rabbit central nervous tissue, it is known that the glycine taken up into neurons remains as such to a remarkable extent in short term incubation experiments[2,5,9]. In these animals, it is also known that there is an active uptake system for glycine, both in the spinal cord[5] and in the retina[6]. The striking similarity between the distribution of radioactivity seen in the human retina and the rabbit, rat, and guinea-pig retinas strongly suggests that in the human retina there is also a very effective neuronal uptake system for glycine. As in the lower animals, there are good reasons for believing that neurons putatively being glycinergic are to be sought among the ones actively taking up glycine[6,9]; in the human retina, certain amacrines are thus possibly glycinergic. As far as the retina can be taken as a model of the situation in the whole brain, the experiment suggests that glycine may be a neurotransmitter also in the human central nervous system[14].

Résumé. L'accumulation de ^{3}H-glucine de la rétine humaine a été étudiée autoradiografiquement. Comme chez les animaux inférieurs, la radioactivité s'accumulait surtout dans la couche plexiforme interne, dans la couche des filaments nerveux et dans des cellules ayant la position des amacrines. Les résultats doivent indiquer que la glucine peut être neurotransmetteuse inhibitive dans l'espèce diffuse des cellules amacrines de l'œil humain et également dans d'autres cellules du système nerveux central de l'homme.

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[13] B. Ehinger, unpublished.

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Sleep in Parabiosis

The nature of sleep has been a topic of interest, and recent sleep research has gradually clarified the sleep-inducing mechanism. Factors involved in induction of sleep phenomena are in general classified as neural and humoral. From the study of Matsumoto and Jouvet[1], monoamines in the brain are considered to be the potent humoral factors.

There are reports of other factors – sleep-producing substances – such as Pieron's classical report on 'hypnotoxin', and recent reports on unidentified 'dialyzable sleep-promoting material'[2–4]. However, these materials were demonstrated by unphysiological procedures, such as restricted condition, crossed-circulation, puncture, injection etc, and recently Ringle and Herndon[5] failed to obtain these sleep-inducing dialysates from sleep-deprived rabbits.

Alekseeva[6] studied pairs of Siamese twins under physiological conditions, and could find no evidence for involvement of humoral factors in sleep mechanisms, since one twin slept while the other was awake. Due to the status of knowledge on sleep at the time of her work, she only examined slow-wave sleep (SS), which is greately affected by neural factors, including behavior and higher nervous activity, and did not examine paradoxical sleep (PS).

The present study was to see whether SS and PS appeared synchronously in parabiotic rats. It is reasonable to presume that sleep would become more synchronized in proportion to the degree of homeostasis between the parabiotic rats.

Method. Male, Wistar strain rats, weighing 150 to 250 g, from different litters were connected parabiotically by a modification of the Bunster and Meyer[7] method under Nembutal narcosis. In the early stage of experiments a parabiotic union was performed 7 to 10 days after operation for polygraphic recording on a single rat, but later the order of the procedure was reversed. Electrodes were implanted on each parabiotic rat under ether anesthesia as described in our previous report[8]. 70 pairs were operated twice, of which 32 pairs died within 3 days after the last operation. The synchronization of sleep in 38 healthy parabiotic pairs with chronically implanted electrodes were compared with those in 25 control pairs united by their skins only.

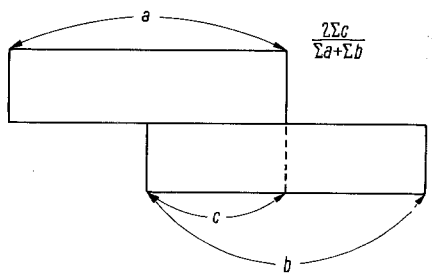

Fig. 1. Interpretation for the synchronization rate.

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The synchronization rate was calculated as: $2\Sigma c/(\Sigma a + \Sigma b)\%$, where a is the time of a single sleep period in 1 rat, b is that of the partner rat, and c is the time duration of overlapped episode of each sleep in the 2 rats (Figure 1). Thus, if all the PS periods of 1 rat appeared synchronously with that of its partner, the synchronization rate of PS should be 100%. The rates of parabiotic and control pairs were compared for up to 15 days after union, because physical separation of the control pairs inevitably occurred in less than 15 days. Polygrams were recorded for 8–10 h a day (Figure 2).

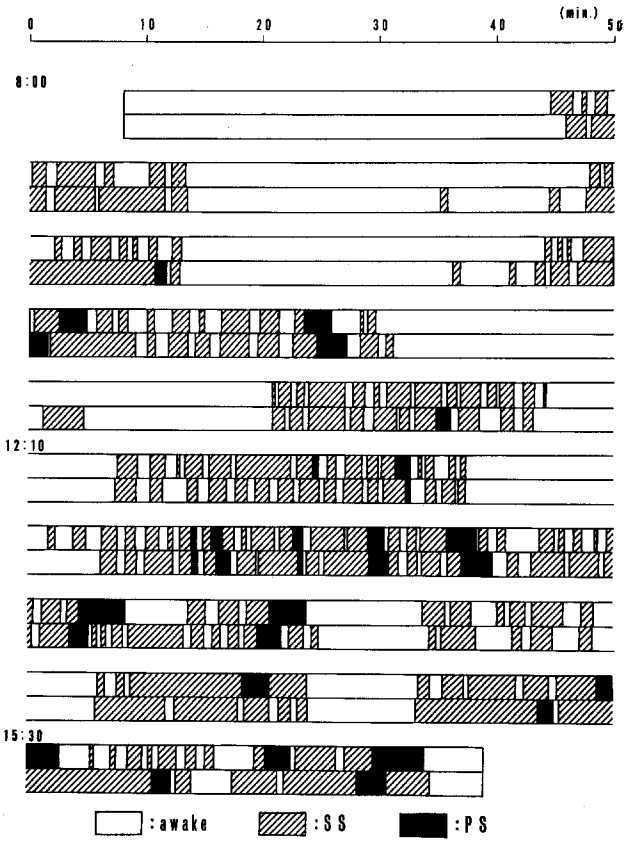

Fig. 2. Relation of wakefulness and sleep in a parabiotic pair (11th days after union). Each Σa, Σb and Σc is 33′24″, 22′46″ and 8′40″ for PS, 155′42″, 202′36″ and 122′24″ for SS. The synchronization rate is 30.9% for PS, 68.3% for SS according to the formula. Each PS/TT is 7.0%, 4.7% and SS/TT 32.3%, 42.1% (TT = 481′). Synchronous appearance of PS are observed in groups clearly during 13 h and 14 h 20 min.

The experiment was divided into seven 2-day-periods after the parabiotic union (2–3, 4–5, 14–15 days) and when the percentage of total recording time occupied by PS (PS/TT) in the 1 rat was less than 3%, the result was disregarded as abnormal. In almost all cases rats showed low PS/TT values on the day before death.

Results. The synchronization rates of parabiotic rats were higher than those of control rats in all experimental periods, and the difference in the synchronization rates of PS were statistically significant in 2–3 ($P < 0.02$), 4–5 ($P < 0.01$), 10–11 ($P < 0.05$) days after the union. The difference in the synchronization rates of SS was not significant in these periods. But the average values of all the experimental periods showed significant difference at levels of $P < 0.01$ for SS and $P < 0.001$ for PS (Figure 3).

It is not surprising that the synchronization rates of SS is higher than that of PS, because in normal individual rats SS occupies 49.6% (SS/TT) of the day and PS only 8.1% (PS/TT)[8]. It should be noted that under parabiotic conditions change from wakefulness to SS is mutually disturbed by the behavior of the 2 animals, while that from SS to PS is not.

In 1 parabiotic pair which survived for 81 days, the average of the synchronization rates in 64, 70 and 77th days after union was 74.6% for SS, and 30.04% for PS (Figure 3). These values are about the same as those obtained within 15 days.

It is noteworthy that in the control pairs synchronous periods of PS between 2 rats generally occurred sporadically, while in the parabiotic pairs these periods occurred in groups as shown in Figure 2. From these observations it seems likely that humoral homeostatic conditions for inducing, viz. initiating and promoting PS are readily attained in parabiosis. It has been well established by other authors[9–11] that in parabiotic rats about 1% of the total blood of each rat passes into the other animal per minute. Thus it seems unlikely that in the parabiotic condition a bloodborne sleep-inducing factor would be transferred from 1 animal to the other as quickly as in crossed-circulation experiments.

In conclusion, homeostatic conditions may be obtained for induction of paradoxical sleep. Humoral factors, including physicochemical factors, seems to be involved in sleep mechanisms and are more important in induction of PS than in induction of SS.

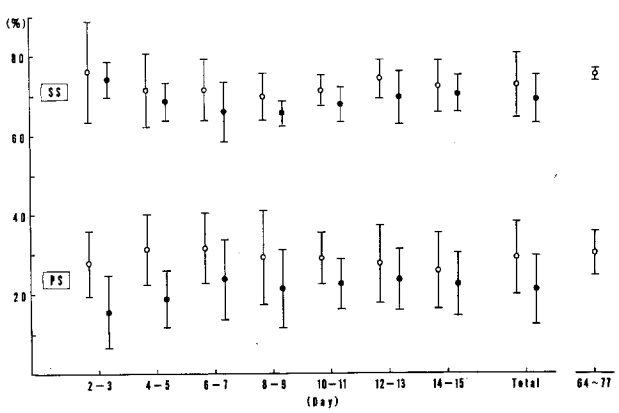

Fig. 3. Comparison of the synchronization rates in parabiotic (○) and control pairs (●). Rates are given as means ± the standard deviation and significance is calculated by the Student's t-test (see the text).

Résumé. Le taux de la synchronization de la phase de sommeil chez le rat parabiotique est plus élevé que chez le rat de controle, particulièrement en cas de sommeil paradoxal ($P < 0.001$). Ces resultats indiquent que le facteur humoral joue un grand rôle dans le déclenchement et l'anticipation du sommeil paradoxal.

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