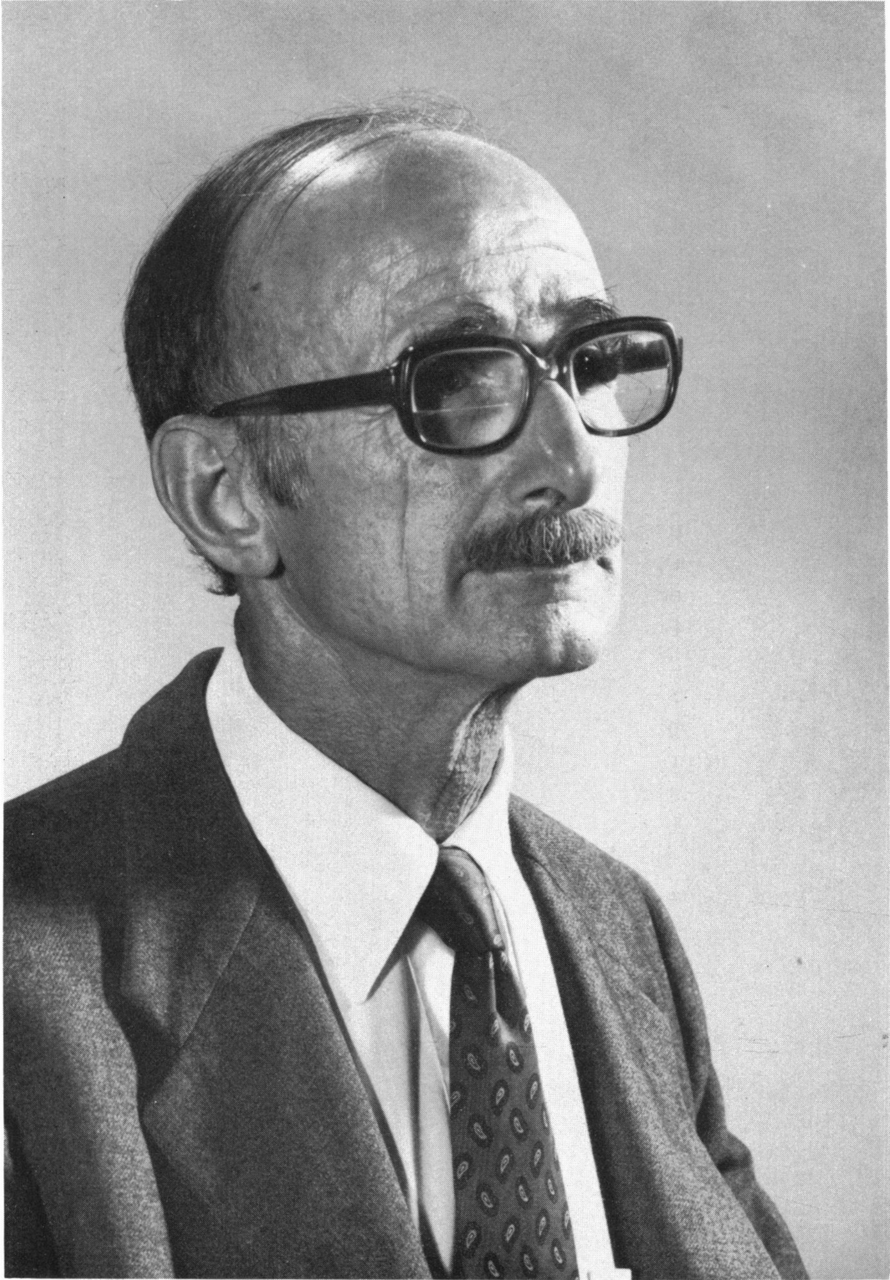

J. Physiol. (1983), **336**, *pp.* 1–11
With 4 *text-figures*
Printed in Great Britain

BAYLISS–STARLING MEMORIAL LECTURE (1982)

INDUCTION OF SLEEP BY MURAMYL PEPTIDES

J. R. PAPPENHEIMER

From the Harvard Medical School, Boston, MA 02115, *U.S.A.*

Memories of Bayliss and Starling were fresh in the minds of my teachers in the 1930s. My tutor at Harvard College was Jeffries Wyman who had worked here with A. V. Hill in the 1920s; some of you will recall the Levin–Wyman model of the visco-elastic properties of muscle (Levin & Wyman, 1927). It was through Jeffries Wyman that I came to know Bayliss's *Principles of General Physiology* which remains the most treasured volume on my physiological shelf. Sixty years of explosive developments in science have left most of the contents out of date but its unique blend of physics, chemistry, general biology and sense of history continues to represent the best of physiology. In 1924 Sir William was too ill to complete the ongoing revision of his book and a committee was formed under the leadership of A. V. Hill to complete the revision and to see the fourth edition through the press. In an addendum to the preface A. V. Hill described work of the committee as '...witness of the affection which (Sir William) inspired in all who had the good fortune to come within the magnetic field of goodness and kindness which surrounded him'.

In 1936 I took Part II Physiology at Cambridge where I became involved in research with Frank Winton on the osmotic work of the kidney. Soon I was enrolled as a research student with Winton and we were joined by Grace Eggleton, initially in Cambridge and subsequently at University College where Winton moved to the Chair of Pharmacology in 1938. We became a close-knit family, both in the lab and out, and of course this included Leonard Bayliss who married Grace Eggleton during this period. Under Frank Winton's tutelage I learned some of the techniques developed by Starling for perfusion of isolated mammalian organs. The 1894 paper by Bayliss & Starling on the relations between venous pressure and capillary pressure and the 1896 paper by Starling on *Absorption of fluid from the connective tissue spaces* were the foundations for my own contributions to physiology of the capillaries some 50 years later. Given this history, I can perhaps claim to be one of the many scientific grandchildren of Bayliss and Starling but in any case I am deeply sensible of the honour associated with an invitation to give a Bayliss–Starling Memorial Lecture. My lecture will be mainly on current research but I will introduce it with a reminiscence.

My thesis examiners for the Cambridge degree were E. B. Verney of Cambridge and Sir Charles Lovatt Evans from University College; both were pupils of Starling's. Arrangements were made to conduct the examination in a Pub in Oxford during meetings of the Society. Fortunately for me, the time set for examination conflicted

* A review lecture delivered at University College London in March 1982.

with a communication I was scheduled to give at the meetings and you can all guess what the priorities would have to be under such circumstances. The examination was postponed indefinitely but perhaps now, 43 years later, it has been resurrected in the form of a Bayliss–Starling Lecture. At any rate, my 1982 thesis is entitled *Induction of slow-wave sleep by muramyl peptides* and I have to confess that this time two years ago I had never heard of muramyl peptides. They are subunits of polymeric peptidoglycans in the cell walls of bacteria and you may well wonder how such prokaryocytic molecules could be related in any way to function of the central nervous system in higher animals and specifically to regulation of sleep and body temperature. This unexpected relationship is the subject of my lecture and I will present it in three main parts. *Firstly*, I will review very briefly the main steps leading to isolation and chemical characterization of sleep-promoting factor obtained from human urine. *Secondly*, I will describe the physiological effects of sleep factor in rabbits and cats. *Finally*, I will discuss the somnogenic and pyrogenic effect of certain synthetic muramyl peptides which are chemically related to urinary sleep factor.

I. STEPS LEADING TO ISOLATION AND CHARACTERIZATION OF SLEEP FACTOR

About 15 years ago Miller, Goodrich & Pappenheimer (1967) repeated with variations some early experiments by Legendre & Pieron (1913) showing that intracisternal injections of c.s.f. taken from sleep-deprived animals can induce sleep in recipient animals. We used goats as donors because we had been working on central chemical control of breathing in these animals and had found that the shape and thickness of occipital bone in goats made possible implantation of guide tubes over the cisterna magna for repeated withdrawl of c.s.f. without anaesthesia. We used rats as recipients because we thought that their day–night pattern of sleep and locomotor activity might make biological assays for sleep relatively easy. Fig. 1 is taken from our first detailed paper on the Pieron Phenomenon (Fencl, Koski & Pappenheimer, 1971). The essential observation was that rats receiving sterile c.s.f. from sleep-deprived goats had more slow-wave sleep (s.w.s.) and were less active at night than the same rats which received control c.s.f. from the same goats. Observations similar to these have recently been made by Borbely & Tobler (1980) who used rats rather than goats as donor animals. Of course, there was great variability between individual rats and we had to make many measurements on each of several rats in order to prove a significant sleep effect. Nevertheless, by 1971 we were convinced that the phenomenon was real and its implications seemed so intriguing that we set out to isolate and identify the sleep-promoting materials. By 'we' I include several colleagues whom I shall mention as we go along but especially Professor Manfred Karnovsky who combines the professional skills of modern biochemistry with sympathetic understanding of old-fashioned physiology and physiologists. At first we tried to isolate sleep factor from c.s.f. of sleep-deprived goats and for this purpose established a colony of twenty-five goats, each provided with a guide tube implanted in occipital bone to facilitate withdrawal of cisternal fluid (Pappenheimer, Koski, Fencl, Karnovsky & Krueger, 1975). Over a period of three years we collected about 6 l. of c.s.f. from these animals. We now know that there is far too little sleep factor in 6 l. of c.s.f. to permit systematic chemical studies. Nevertheless, from this early work on c.s.f.

we learned that sleep factor had a molecular weight of something less than 1000 Daltons, that it came out slightly ahead of sucrose during gel filtration through Sephadex columns and that it was inactivated by proteolytic enzymes. This 'smelled' like a small peptide and so we proceeded to extract Factor S from brains of sleep-deprived animals using methods described by others for small peptide hormones.

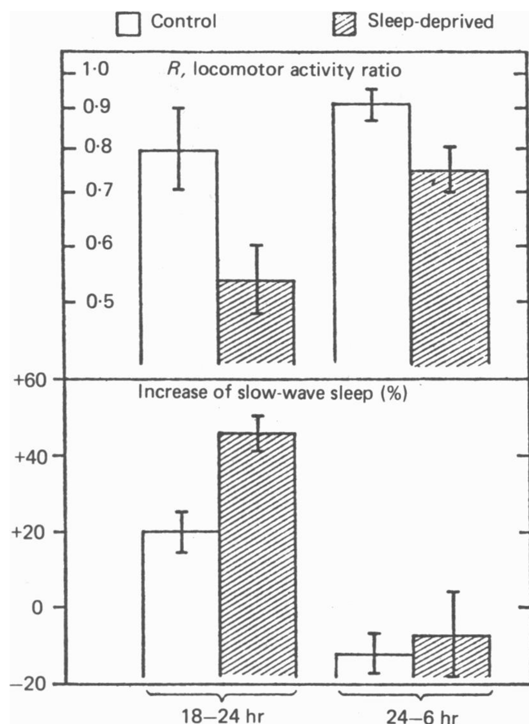

Fig. 1. Simultaneous measurements of nocturnal locomotor activity (upper panel) and increase of slow-wave sleep (lower panel) in rats following intraventricular infusions of 0·2 ml. c.s.f. from control and from sleep-deprived goats. Means ± S.E. of means from seven rats. From Fencl *et al.* (1971).

We used brains from sleep-deprived goats or sheep and later from rabbits, first 100 rabbits then 3000 rabbits and eventually 15,000 rabbits. Even this large-scale attempt ended in partial failure because the yield was too small to permit definitive chemical studies. Nevertheless, Dr James Krueger and Professor Karnovsky were able to develop some unique purification procedures which proved to be effective when we moved to the next source of Factor S, namely human urine (Krueger, Bacsik & García-Arrarás, 1980). The fact that a single dose of Factor S induced sleep for several hours suggested that the molecule might be as stable *in vivo* as it seemed to be *in vitro* and if this were the case it might be absorbed into sagittal sinus blood along with c.s.f. and eventually be excreted in the urine.

From about 5000 l. of human urine we obtained some 30 μg of a relatively pure glycopeptide which induced sleep in rabbits, rats and cats. The yield was estimated from analyses for amino acids and amino sugars and from mass spectral analyses.

The development of purification procedures involved assays for sleep-inducing activity in hundreds of samples generated by ion exchange, gel filtration, paper chromatography, electrophoresis and other more specialized techniques for purifying the unknown. Each assay required 6 hr of recording on two or more rabbits fitted with implanted guide tubes and e.e.g. electrodes. This part of the research took several years and there was very little to show for it until there was enough pure product to analyse chemically. Mild hydrolysis of the final product yielded muramic acid, alanine, glutamic acid and diaminopimelic acid in molar ratios 1:2:2:1. There were also variable amounts of glycine and glucosamine but the biological activity correlated best with the first four constituents and we provisionally assigned these to the structure of urinary Factor S (Krueger, Pappenheimer & Karnovsky, 1982). More recent studies by mass spectrometry confirm this composition but indicate the presence of an additional component to give a total molecular weight between 921 and 922 Daltons.

Muramic acid and diaminopimelic acid are constituents of cell walls in bacteria and there are no known synthetic pathways for either of these molecules in animals. We are therefore confronted with problems concerning the origin and significance of the sleep-promoting factor. I will return to these problems after discussing the somnogenic and pyrogenic effects of synthetic muramyl peptides but now it is time, after this brief overview of history, to describe in more detail the physiological effects of sleep factor derived from sterile c.s.f., from acid–acetone extracts of fresh brain or from urine.

II. PHYSIOLOGICAL EFFECTS OF SLEEP FACTOR IN RABBITS AND IN CATS

I will start with rabbits because laboratory rabbits have relatively simple and stable patterns of sleep. Rabbits sleep about 40% of each hour during the daytime with an S.D. of only ±7%. In male rabbits the rapid eye movement component of sleep (r.e.m.) is negligible so we have only to consider regular episodes of slow-wave sleep (s.w.s.). The sleep of rabbits consists of many short episodes, each episode lasting up to 10 min but mostly in the 1–4 min range. Each episode involves generation of fully developed slow waves of a characteristic amplitude which can be measured as the r.m.s. voltage of the rectified 0·5–4 Hz band of the e.e.g. In the awake state the mean rectified slow-wave component is generally around 10 μV and it increases to about 40 μV during each episode of normal sleep. In any one rabbit these differences in slow-wave voltages between sleep and wakefulness are quantitatively repeatable within about 10% for weeks or even for months.

Intraventricular infusions of sleep factor, whether this be derived from c.s.f., brain or urine, is followed by increase in the number of episodes of sleep which last longer than five minutes and with an increase in the amplitude of slow waves during sleep. Typical assays showing maximal sleep responses to intraventricular infusions of Factor S purified from brain extracts or from urine are shown in Fig. 2. The data summarize assays on eight rabbits. Note that the sleep effect does not begin for 1–2 hr after the intraventricular infusion has stopped. The excess sleep may continue for 8–12 hr but for routine assays, such as those shown in Fig. 2, the recordings are stopped after 6 hr. The maximal response is about 70% of s.w.s. for 6–8 hr. The sleep appears

›o be natural in that the animals can easily be aroused and even during a maximal ·esponse they wake up spontaneously from time to time to eat, drink or groom. The ·esponse is roughly dose-dependent and a maximal response is obtained with a dose ›f 10–20 p-moles (10–20 ng) in an adult rabbit weighing 3–4 kg. The maximal ·esponse is about five times greater than the standard deviation of the controls so ·hat two to four rabbit assays are sufficient to establish the presence or absence of !0 p-moles or more of sleep factor in any given unknown fraction.

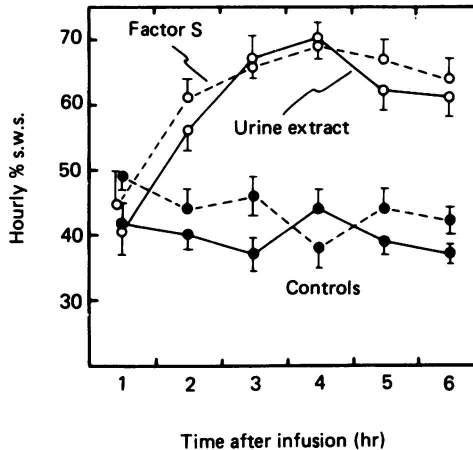

Fig. 2. Time course of effects of Factor S on slow-wave sleep (s.w.s.). O—O effects of urine extracts purified through CM-Sephadex ion exchange and G10-Sephadex gel filtration steps. Means ± s.e. of means of thirteen assays on ten rabbits. Each rabbit received a dose equivalent to 20–90 ml. of original urine (mlu). ●—● control values on same rabbits. O---O effects of acid–acetone extracts of brains from sleep-deprived rabbits. Extracts were purified by the same ion exchange and gel filtration steps used for urine. Means ± s.e. of means of ten assays on eight rabbits. Each rabbit received a dose equivalent to 6 g of original brain (gbe). ●---● controls on same rabbits. Modified from Krueger *et al.* (1980). Courtesy of the American Physiological Society.

So much for rabbits. I turn now to the sleep of cats which is more complex because :ats have at least two stages of slow wave sleep (Ursin, 1968) and a substantial :omponent of r.e.m. sleep. Also, the sleep of cats is more easily disturbed by ɜnvironmental factors and by changes of body temperature induced by pyrogens. Dr García-Arrarás has studied the effects of urinary Factor S in each of five cats which were kept in the laboratory for several months under controlled environmental :onditions (García-Arrarás, 1981). He took repeated 32 hr recordings from each cat ıfter intraventricular infusions of artificial c.s.f. as control or after highly purified ırinary sleep factor. The results are shown in Fig. 3. The cats slept an average of 4·9 ± 0·2 hr (s.w.s.) during the first 12 hr after infusions of control fluid. After receiving 100 p-moles of Factor S the corresponding value was 6·9 ± 0·5 hr with a peak effect of 70 % s.w.s. per hour 4 hr after the infusion. Most of this induced s.w.s. was in the form of Stage II high amplitude s.w.s. There was no rebound of wakefulness to make up for the added sleep induced by Factor S, at least in the 32 hr of recording. In contrast, r.e.m. sleep was partially inhibited during the first 4 hr but this was made

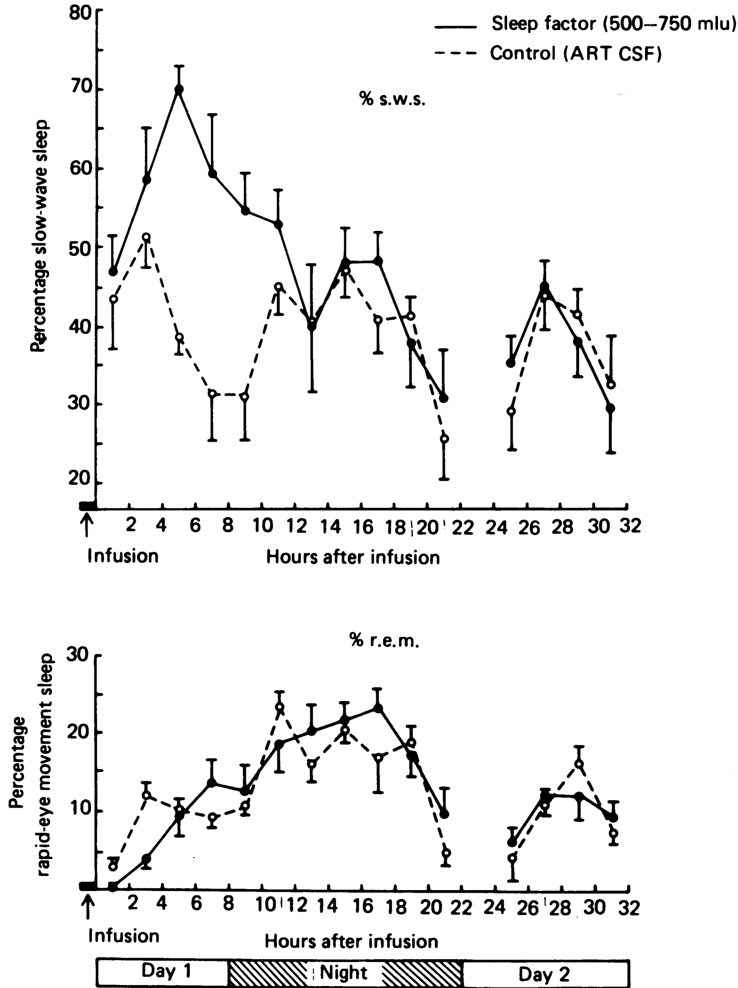

Fig. 3. Slow-wave sleep (top panel) and rapid-eye-movement sleep (bottom panel) of cats were measured after intraventricular infusions of 500–750 ml original urine equivalents dissolved in 0·3 ml artificial c.s.f. (●—●) and compared to control infusions of artificial c.s.f. (O---O). Average values were obtained for each of five cats; these include thirteen infusions of urinary sleep factor and ten infusions in artifical c.s.f. Means ± S.E. of mean of these averaged values are shown. Animals were taken out of cage for 10–21 min on the 8th hour after infusion and for about 1 hr on the 22nd hour after infusion. Modified from García-Arrarás (1981). Courtesy of the American Physiological Society.

up in subsequent hours so that there was no net change of r.e.m. as there was for s.w.s.

Sleep responses to purified Factor S can also be elicited by micro-injections into localized regions of the brain. Fig. 4 is a diagrammatic summary showing results of injecting 1 μl. containing about 10 p-moles of Factor S into histologically identified regions of the brain in rabbits. Positive sleep responses were obtained at eight out of fifty-two injection sites. The eight sites were located between the basal forebrain

and the mesodiencephalic junction. According to Sterman and his colleagues, low frequency electrical stimulation of this region induces s.w.s. in cats (Sterman & Clemente 1962) and electrolytic lesions produce insomnia (McGinty & Sterman, 1968).

I turn now to the effects of some synthetic muramyl peptides on sleep and fever.

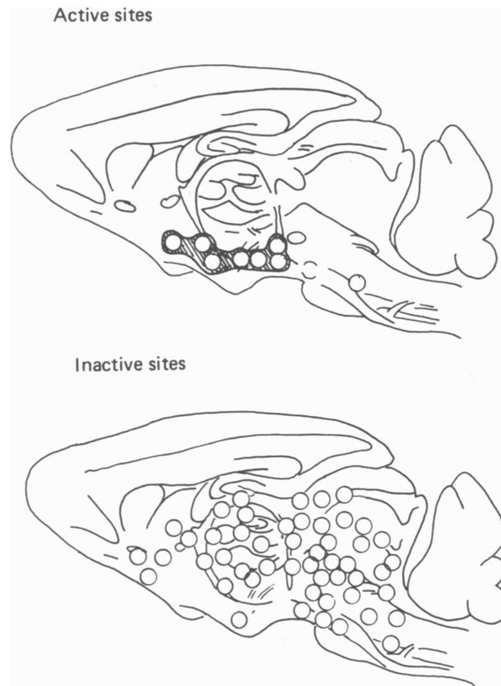

Fig. 4. Locus of action of urinary sleep factor in rabbits as determined by micro-injections of 1 μl. containing 10 p-moles of the purified muramyl hexapeptide. Seven of the eight active sites were located as shown between the basal forebrain and the mesodiencephalic junction. Modified from García-Arrarás & Pappenheimer (1983). Courtesy of the American Physiological Society.

III. EFFECTS OF SYNTHETIC MURAMYL PEPTIDES

We have already seen that sleep factor isolated from urine is a muramyl peptide which resembles some of the products obtained by enzymatic digestion of bacterial cell wall peptidoglycans. It has been known for many years that the bacterial peptidoglycans can greatly enhance the yield of antibodies raised in response to any antigen. The simplest synthetic analogue which has this property is a muramyl dipeptide, *N*-Ac-muramyl-L-alanyl-D-isoglutamine, abbreviated MDP (Ellouz, Adam, Ciorbaru & Lederer, 1974). Much of the chemical and immunological work on synthetic muramyl peptides has been done by Professor E. Lederer at the C.N.R.S. for Biochemistry at Gif-sur-Yvette (see Lederer, 1980 for review) and by Professor L. Chedid at the Institut Pasteur. They have very kindly supplied us with several synthetic muramyl peptides.

We soon found that intraventricular infusions of 75–150 p-moles of MDP in rabbits

induced prolonged excess high amplitude s.w.s. (Table 1) which closely resembled the excess sleep induced by 5–10 p-moles of the urinary mulamyl peptide. Intravenous, intraperitoneal and even oral administration of MDP induced effects similar to those shown here for the intraventricular route. However, the intravenous dose required is about 10,000 fold greater than the intraventricular dose and the oral dose is about one million times greater.

TABLE 1. Effects of MDP and MDP-lys during 6 hr after intraventricular infusions of 75–150 p-moles

% s.w.s.		Amplitude of slow waves (μV)		
Control	Experimental	Control	Experimental	ΔT brain (°C)
39±1·7	60±3·6	40±5	65±11	1·9±0·2

Values are mean ± S.E. of mean in twelve rabbits.

In a few experiments on rabbits I have infused MDP intraventricularly for 24 hr at the rate of 0·5 p-mole per minute. The animals slept an average of 60 % of the time throughout these long infusions and they reverted to their nomal 35–40 % soon after the infusion stopped.

Like subunits of bacterial peptidoglycans, MDP is a pyrogen as well as an immunostimulant. Chedid and his colleagues at the Institut Pasteur (Riveau, Masek, Parant & Chedid, 1980) have found that MDP acts as a pyrogen in two ways. When given systemically it stimulates leucocytes to release endogenous pyrogen, a small protein. However, MDP can also act directly on central neurones without the release of leucocytic pyrogen; intraventricular injection of as little as 50 p-moles of MDP in a rabbit will induce a 1–2 °C rise in body temperature. In our 24 hr infusion experiments the body temperature remained high throughout the infusion. In rabbits the fever does not seem to interfere with the sleep inducing effect but in cats the response to MDP is more complicated. During the chill phase of the pyrogenic response the cats appear to be unable to enter Stage II of s.w.s. and r.e.m. is suppressed entirely during this period which may last 3–4 hr. Once the fever has been established and during recovery from fever the cats sleep for long periods in deep s.w.s.

Of the various derivatives tested so far only MDP-lysine has had somnogenic effects comparable to those of MDP or to the urinary muramyl peptide. Stereoisomers of MDP containing D-alanine or L-isoglutamine (MDP-DD and MDP-LL) are non-pyrogenic and they also failed to affect sleep, even in doses up to 1000 p-moles.

Is it possible to separate the pyrogenic effects of muramyl peptides from their sleep-promoting effects? This question is of obvious importance to the pharmaceutical industry but the answer does not appear to be a simple one, at least at the present time. We have tested several synthetic muramyl peptides which are immuno-stimulants but which are not pyrogenic but none of these have induced sleep. The muramyl peptide isolated from urine gives equivocal answers; in some rabbits it has induced sleep without significant fever but in others it induced fever as well as sleep. Dr Krueger has recently found that the drug Acetaminophen will block or attenuate the pyrogenic effects of intravenously administered MDP while leaving its somnogenic action intact. The time course of the somnogenic effect is often quite different from

that of the pyrogenic effect and we have already seen that in cats sleep is inhibited, rather than enhanced, during the initial chill phase of the pyrogenic response. Thus, muramyl peptides can affect temperature and sleep independently but more work needs to be done to define the conditions for complete separation of the two effects.

IV. CONCLUSIONS AND SPECULATIONS

With this background of facts I will now enter the final phase of this lecture to discuss in a speculative way what the relations may be between muramyl peptides and the regulation of sleep and temperature in mammals.

It seems obvious that high affinity receptors for muramyl peptides must be present in brain, presumably in the anterior hypothalamus for temperature regulation and in the more caudal regions we have designated for the somnogenic effects. If high affinity receptors are there we should look for endogenous agonists and we may ask if the muramyl peptide isolated from urine is in fact a natural endogenous sleep-inducing neuromodulator. Its physiological effects and at least some of its physico-chemical properties are the same as those of the sleep factor which accumulates in the c.s.f. in the course of sleep deprivation. Synthetic ^{14}C-labelled MDP, injected intravenously, is excreted rapidly and quantitatively in the urine (Parant, Parant, Chedid, Yapo, Petit & Lederer, 1979) so it is not unreasonable to suppose that a muramyl peptide might originate in brain, diffuse to the c.s.f., enter sagittal sinus blood and reach the urine unaltered.

On the other hand, it is generally believed by biochemists that muramic acid and diaminopimelic acid cannot be synthesized by animal cells and so we must recognize the possibility that the muramyl peptide isolated from urine might be a breakdown product from contaminating bacteria. We cannot exclude this possibility at present but there are some strong arguments against it. Many preparations of urinary Factor S have been made using a variety of different methods of collecting and storing urine and all of these have provided approximately the same yield of Factor S; this would not be expected if the muramyl peptide derived from adventitious bacterial contamination. In the case of sleep factor derived from brain there was little chance for contamination because the fresh brains were rapidly immersed in cold acid–acetone and all subsequent steps of purification were controlled by biological assay of reagent blanks. Yet the brain material behaved similarly to the urinary material in every chromatographic or ion exchange system we employed. Sleep-promoting material derived from sterile c.s.f. was not so well characterized chemically but its physiological properties and its behaviour on G-10 Sephadex columns were indistinguishable from the peptide derived from urine. These facts argue against bacterial contamination.

An alternative hypothesis is that subunits of bacterial peptidoglycans are normally absorbed in the gut and that small amounts reach the brain for incorporation into neuromodulators. Free diaminopimelic acid is in fact present in body fluids, including urine (Krysciak, 1980) and Red Cross blood plasma. Similarly, muramic acid is present in hydrolysates of sleep factor purified from brain (Krueger *et al.* 1982).

Finally, we must leave room for a third possibility, namely that muramic acid and diaminopimelic acid are actually synthesized by mammalian tissues as they are in bacteria.

Most of these questions about the origin of urinary muramyl peptide can be

answered by experiment but new tools must be fashioned for the job such as the production of antibodies to muramyl peptides, receptor assays using labelled muramyl peptides and so on. By way of *hypothesis*, subject to change as more information accrues, I suggest that the muramic acid and diaminopimelic acid found in mammalian sleep factor derive from bacteria taken in with food and may be regarded as akin to any of the essential amino acids or vitamins which cannot be synthesized by mammalian cells. In stating this hypothesis so boldly let me remind you of the Introduction to Bayliss's *Principles* in which he says '...it is better to hold a well-understood and intelligent opinion even if it is wrong, than to be content with a (muddle-headed) mixture of conflicting views...' In the present instance the issues at stake are challenging to biochemists and immunologists as well as to physiologists and I suspect it will not be long before our hypothesis is demolished, modified or perhaps even substantiated. In the meantime we can conclude with the facts, namely that certain muramyl peptides, whether endogenous or exogenous, natural or synthetic, can act directly on the central nervous system in picomole quantities to induce prolonged excess sleep of a type which is qualitatively indistinguishable from natural deep slow-wave sleep.

Much of the recent work on which this lecture is based was carried out in collaboration with Dr James M. Krueger, Professor Manfred L. Karnovsky and Dr Jose García-Arrarás. I am indebted to them for allowing me to present results which have not previously been published in the form of original articles. It is for this reason that some of the primary data in support of the conclusions were shown as illustrations at the time of the lecture but are not included in the present text. It is our expectation that the detailed data and figures shown during the lecture will be published in primary journals in the usual way.

Mass spectrometry studies mentioned on p. 4 were conducted by members of the NIH-sponsored Mass Spectrometer Facility at MIT, who have kindly given us permission to quote this result of their preliminary work.

I wish to acknowledge the long term support of this work by the American Heart Association as well as recent support from the Office of Naval Research (Contract No. 0014-77-C-0774).

REFERENCES

BAYLISS, W. M. & STARLING, E. H. (1894). Observations on venous pressures and their relationship to capillary pressures. *J. Physiol.* **16**, 159–202.

BORBELY, A. & TOBLER, I. (1980). The search for an endogenous 'sleep-substance'. *Trends in Pharmacological Sciences* **1**, 357–359.

ELLOUZ, F., ADAM, A., CIORBARU, R. & LEDERER, E. (1974). Minimal structural requirements for adjuvant activity of bacterial peptidoglycan derivatives. *Biochem. biophys. Res. Commun.* **59**, 1317–1325.

FENCL, V., KOSKI, G. & PAPPENHEIMER, J. R. (1971). Factors in cerebrospinal fluid from goats that affect sleep and activity in rats. *J. Physiol.* **216**, 565–589.

GARCÍA-ARRARÁS, J. (1981). Effects of sleep-promoting factor from human urine on sleep cycle of cats. *Am. J. Physiol.* **241**, E269–274.

GARCÍA-ARRARÁS, J. & PAPPENHEIMER, J. R. (1983). Site of action of sleep-inducing muramyl peptide isolated from human urine; microinjection studies in rabbit brains. *J. Neurophysiol.* (in the Press).

KRUEGER, J. M., BACSIK, J. & GARCÍA-ARRARÁS, J. (1980). Sleep-promoting material from human urine and its relation to Factor S from brain. *Am. J. Physiol.* **238**, E116–123.

KRUEGER, J. M., PAPPENHEIMER, J. R. & KARNOVSKY, M. L. (1982). The composition of sleep-promoting factor isolated from human urine. *J. biol. Chem.* **257**, 1664–1669.

KRYSĆIAK, J. (1980). Diaminopimelate in mammalian urine. *Folia biol., Kraków* **28**, 47–51.

LEDERER, E. (1980). Synthetic immunostimulants derived from the bacterial cell wall. *J. medicinal Chem.* **23**, 819–825.

LEGENDRE, R. & PIERON, H. (1913). Recherches sur le besoin de sommeil consécutif à une veille prolongée. *Z. allg. Physiol.* **14**, 235–262.

LEVIN, A. & WYMAN, J. (1927). The viscous elastic properties of muscle. *Proc. R. Soc.* B **101**, 218–243.

MCGINTY, D. J. & STERMAN, M. B. (1968). Sleep suppression after basal forebrain lesions in the cat. *Science, N.Y.* **160**, 1253–1255.

MILLER, T. B., GOODRICH, C. A., & PAPPENHEIMER, J. R. (1967). Sleep-promoting effects of cerebrospinal fluid from sleep deprived goats. *Proc. natn. Acad. Sci. U.S.A.* **58**, 513–517.

PAPPENHEIMER, J. R., KOSKI, G., FENCL, V., KARNOVSKY, M. L. & KRUEGER, J. M. (1975). Extraction of sleep-promoting Factor S from cerebrospinal fluid and from brains of sleep-deprived animals. *J. Neurophysiol.* **38**, 1299–1311.

PAPPENHEIMER, J. R. (1979). 'Nature's Soft Nurse': A sleep-promoting factor isolated from brain. *Johns Hopkins Med. Jl.* **145**, 49–56.

PARANT, M., PARANT, F., CHEDID, L., YAPO, A., PETIT, J. F. & LEDERER, E. (1979). Fate of the synthetic immunoadjuvant muramyl dipeptide (^{14}C-labelled) in the mouse. *Int. J. Immunopharmac.* **1**, 35–41.

PARANT, M., RIVEAU, G., PARANT, F., DINARELLO, C. A., WOLFF, S. M. & CHEDID, L. (1980). Effect of Indomethacin on increased resistance to bacterial infection and on febrile responses induced by muramyl dipeptide. *J. infect. Dis.* **142**, 708–715.

RIVEAU, G., MASEK, K., PARANT, M. & CHEDID, L. (1980). Central pyrogenic activity of muramyl dipeptide. *J. exp. Med.* **152**, 869–877.

STERMAN, M. B. & CLEMENTE, C. D. (1962). Forebrain inhibitory mechanisms: sleep patterns induced by basal forebrain stimulation in the behaving cat. *Expl Neurol.* **6**, 91–102.

STARLING, E. H. (1896). On the absorption of fluids from the connective tissue spaces. *J. Physiol.* **19**, 312.

URSIN, R. (1968). The two stages of slow wave sleep in the cat and their relationship to REM sleep. *Brain Res.* **11**, 347–355.