positive intermediary potential may be of importance, since it could conceivably explain some actions of this drug, particularly those on the reticular formation described by DEMAAR et al.[7]. In view of these observations, an investigation was undertaken to determine whether β-MSH can antagonize the action of chlorpromazine on the positive intermediary potential of cat spinal cord.

Methods and Materials. Seven decerebrate cats were used in this study. Three of these had spinal transections at L_1. The techniques used to study the positive intermediary potential were modifications of those of LLOYD and MCINTYRE[6]. A complete description is being presented elsewhere[8]. Briefly, it consists in stimulating a spinal rootlet from the last lumbar segment and electronically displaying the activity so evoked in an immediately adjacent dorsal spinal rootlet. Stimuli used in these experiments were maximal, and delivered at a frequency of 0.5 cps (typical settings of the stimulator were 0.2 msec duration and 0.05 V amplitude). Once stimulation was started, it was continued for the duration of the experiment. Decerebration was performed under ether anesthesia which was discontinued at least 1 h prior to the initiation of the experiment. Then the experiment was started only after the positive intermediary potentials had been seen to remain constant for at least 30 min[9].

Results. Similar results were obtained in both decerebrate and decerebrate-spinal cats. The administration of chlorpromazine in doses of 4 to 10 mg/kg resulted in inhibition of the positive intermediary potential. The subsequent administration of β-MSH in doses of 0.14 to 0.25 mg/kg resulted in restoration of the positive intermediary potential towards normal. The time course of this effect followed that described previously regarding modifications of evoked monosynaptic spinal potentials appeared approximately 4 min after injection, reaching a maximum ca. 20 min thereafter, and lasting for more than 30 min. There were no changes in blood pressure or respiration that could be associated with the alterations in positive intermediary potentials.

Conclusions. β-MSH is capable of antagonizing the actions of chlorpromazine probably by acting directly on the cat spinal cord. The observations reported here would be in agreement with the hypothesis that chlorpromazine acts to lower the central excitatory state by modifying the activity of β-MSH in the nervous system[10].

Résumé. Une préparation hautement purifiée de l'hormone mélanophorétique β-MSH inhibe les effets de la chlorpromazine au niveau de la moelle spinale.

W. A. KRIVOY and R. GUILLEMIN

Departments of Pharmacology and Physiology, Baylor University College of Medicine, Houston (Texas USA) and Laboratoire de Morphologie expérimentale et Endocrinologie, Collège de France, Paris (France), August 7, 1961.

[7] E. W. DEMAAR, W. MARTIN, and K. UNNA, J. Pharmacol. exp. Therap. *124*, 77 (1958).

[8] W. KRIVOY, Brit. J. Pharmacol., in press.

[9] The β-MSH used here had been prepared by Armour Laboratories (courtesy Dr. W. WHITE and J. FISHER) according to the methods reported in [1,2].

[10] This work was supported by Research Grant (MY 3477) from the National Institutes of Health of the U.S. Public Health Service.

The Potassium Concentration of Frog Ringer's Solution[1]

Ringer's solution for bathing organs and tissues of the frog *in vitro* is generally made to contain a potassium concentration of 2.5 mEq/l. This concentration is probably based on the average concentration of potassium in frog plasma given by FENN[2] in 1936. However, the potassium concentration of frog plasma varies with the species of frog as well as with a number of other variables. In *R. areolata*, *R. catesbeiana*, and *R. esculenta*, for example, the potassium concentration of the plasma is, respectively, 5.8, 4.8, and 5.1 mEq/l[3]. In contrast, recent determinations of the potassium content of plasma in *Rana pipiens* gave an average value of 1.7 ± 0.2 (S.D.) mEq/l based on 30 pooled samples from 90 frogs[4].

It is well known that individual intact leg muscles of the frog lose potassium when they are placed in oxygenated Ringer's solution which contains no potassium. Conversely, muscles placed in Ringer's solution which contains up to 6–10 mEq K/l, gain potassium. It follows that there is a concentration of potassium between these two extremes at which the average muscle neither gains nor loses potassium. This fact is of great importance when it is desired to study the flux of potassium in both directions across the muscle cell membrane under conditions of steady state.

From data in a previous paper[5], the steady state potassium concentration of the Ringer's solution can be interpolated to be 3.4 mEq/l for paired tibialis anticus longus and iliofibularis muscles of *R. pipiens* (see Figure 2 of ref. [5]). These were muscles of frogs kept at 5°C during the months of October and November. The average weight of each pair of muscles was 175 mg. After dissection, they were placed in Ringer's solution which contained, in m*M*/l, NaCl, 109.5; KCl, 2.0; $MgCl_2$, 1.0; $CaCl_2$, 1.26; NaH_2PO_4, 0.62; Na_2HPO_4, 3.96, and had a pH of 7.4. After 18 h at 5°C, the muscles were transferred to fresh oxygenated Ringer's solution containing 5 m*M* sodium lactate and varying concentrations of potassium and incubated at 20°C for 6 h. Lactate itself reduces the net loss of potassium from frog muscle in Ringer's solution containing 2.0 mEq K/l[5] so the steady state concentration of potassium without lactate would undoubtedly be higher than 3.4 mEq/l for these muscles.

However, potassium concentrations in the range that provides a steady state for potassium in paired tibialis anticus and iliofibularis muscles cause a net gain of potassium in the sartorius muscle of *R. pipiens* (in October and November) under the same *in vitro* conditions. The relationship between the net change in muscle potassium

[1] This investigation was supported by PHS grant no. A-4718 from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

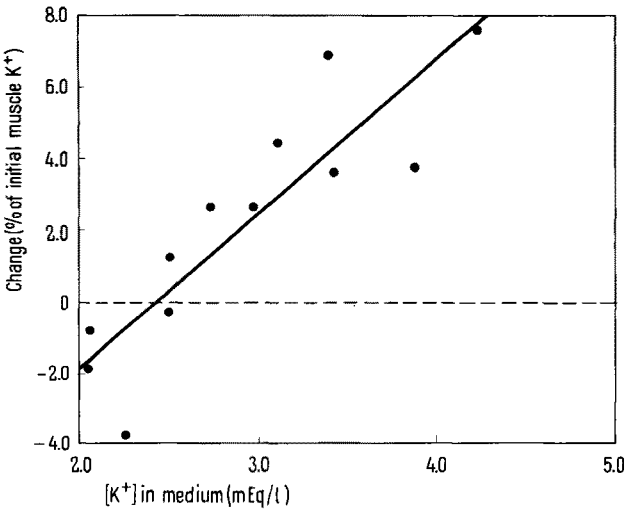
[2] W. O. FENN, Physiol. Rev. *16*, 450 (1936).

[3] P. L. ALTMAN and D. S. DITTMER, *Blood and Other Body Fluids* (Federation of American Societies for experimental Biology, Washington 1961), p. 44.

[4] L. V. GIBBONS and H. M. KAPLAN, Copeia 176 (1959).

[5] D. R. H. GOURLEY, Amer. J. Physiol. *200*, 1320 (1961).

after incubation at 20°C for 6 h is shown as a function of the potassium content of the Ringer's solution (Figure). The calculated regression line drawn in the Figure is given by the equation $y = -10.3 + 4.25\,x$ and the regression coefficient, 4.25, is highly significant ($P < 0.001$). The line passes through the zero change point (shown by the broken line) at a potassium concentration of 2.44 mEq/l, indicating that the fluxes of potassium into and out of the sartorius muscle are equal when the muscle is bathed at 20°C in Ringer's solution containing this concentration of potassium and 5 mM sodium lactate.



The effect of the concentration of potassium in Ringer's solution on the net change in the potassium content of sartorius muscle from *R. pipiens*. NaCl was replaced by KCl so that the sum of Na + K remained constant in each different Ringer's solution. The average weight of each muscle was 98 mg. Zero change in muscle potassium is indicated by the broken line. Each point is the average of determinations in at least 4 muscles. The solid line is the calculated regression line (see text).

It is clear, therefore, that under identical *in vitro* conditions the paired tibialis anticus and iliofibularis muscles and the sartorius muscle require different extracellular concentrations of potassium to maintain a steady state with respect to intracellular potassium. Since the normal concentration of potassium in the plasma presumably provides a steady state for the potassium content of all of these muscles *in vivo*, this *in vitro* phenomenon must be related to the peculiarities of the experimental conditions and to the differences in the size and shape of the muscles. Thus it is concluded that when steady state conditions are important, the concentration of potassium in Ringer's solution must be adjusted to fit the particular experimental conditions and the specific muscles under study.

Résumé. Pour être certain que le dégagement et l'absorption du potassium sont exactement compensés dans un muscle de grenouille étudié *in vitro* dans la solution de Ringer, il faut adapter la concentration du potassium de cette solution aux conditions particulières de l'expérience et tenir compte du fait que le degré de concentration requis varie d'un muscle à l'autre.

D. R. H. GOURLEY

Department of Pharmacology, School of Medicine, University of Virginia, Charlottesville (USA), August 14, 1961.

Threshold for Eliciting the Hippocampal theta-Rhythm by Electrical Stimulation in Tegmentum and Hypothalamus during Cortical Spreading Depression in Rats

Electrical stimulation of the mesencephalic reticular formation elicits a synchronized theta-rhythm in the hippocampus (GREEN[1]). Since this reaction is uninfluenced by bilateral cortical spreading depression (SD) (WEISS and FIFKOVÁ[2]), it is possible to use the hippocampal arousal reaction as an indicator of the excitability of the tegmental reticular formation (RFT) and of the hypothalamus (Hy) during reversible elimination of the cerebral cortex (BUREŠ and BUREŠOVÁ[3]) by SD.

Methods. Bipolar silver electrodes were implanted in the dorsal hippocampus, RFT and Hy (in area hypothal. lat., only in some cases in zona incerta) as was checked histologically (FIFKOVÁ). Rectangular pulses (450 cy/sec, $t < 0.5$ msec, duration of the salves 60 msec) were given at intervals of at least 2 min, but only when spontaneous synchronization in the hippocampus was not present. A constant level of threshold being established for control, unilateral SD was evoked by local application of filter paper (2 × 2 mm) soaked with 25% KCl on the dural surface. After re-estimating the threshold bilateral SD was elicited.

Results. The cortical SD is accompanied always by a rise of thresholds. In no case does the threshold decrease. The increase of threshold was more pronounced in the tegmentum than in Hy (in the latter not significant, see Table). Between the threshold changes in these two areas

	SD ipsilateral to side of stimulation	SD contralateral to side of stimulation	SD bilateral
Tegmentum			
Elevation of threshold (in % of cases)	82.6	80.0	85.8
Number of experiments	11	5	14
Mean elevation of threshold (M) ± mean error (m) %	124.5 ± 6.0*	117.2 ± 8.5*	168.4 ± 15.4*
Significance against control (100%)	$p = 0.01$	not significant	$p = 0.003$
Hypothalamus			
Elevation of threshold (in % of cases)	57.1	20.0	54.5
Number of experiments	7	5	11
Mean elevation of threshold (M) ± mean error (m) %	108.1 ± 4.9*	108 ± 8.0*	109 ± 4.5*
Significance against control (100%)	not significant	not significant	not significant
Significance between values with * marked	$p = 0.05$	not significant	$p = 0.003$

[1] J. D. GREEN, *The Hippocampus*, in *Handbook of Physiology, Neurophysiology* (Washington 1960), II, p. 1373.
[2] T. WEISS and E. FIFKOVÁ, EEG Clin. Neurophysiol. *12*, 841 (1960).
[3] J. BUREŠ and O. BUREŠOVÁ, EEG Clin. Neurophysiol., Suppl. 13, 359 (1960).