the end of the experimental proceedings, the animals were killed by guillotine, the heads being dropped into liquid air and brains removed. After the fresh weight determination, the following regions of brain were dissected: frontal cortex, occipital cortex, hippocampus, caudate nucleus, thalamus and mesencephalic reticular formation. Quantitative analysis of amino acids was carried out chromatographically, using the technique described elsewhere[6,7]. No difference in body weight or in wet brain weight between REM sleep-deprived and control animals was discovered.

Results and discussion. As shown in Tables I and II, the FAA examined were present in different concentrations, lysine threonine and histidine having the lowest levels, while aspartic and glutamic acids the highest. Individual FAA varies to a lesser degree from one region to another. Our results on GABA concentration in cat are higher than those obtained by FAHN and CÔTÈ[9] in Rhesus monkeys. However only 4 regions could be compared: frontal and occipital cortices, thalamus and nucleus caudatus. The difference in results in GABA levels can be explained probably on the basis of species differences rather than assay methods.

From the 11 amino acids observed, 7 changed significantly in certain brain regions under the effect of REM sleep deprivation. The aspartic acid concentrations were significantly elevated in the hippocampus as well as threonine in the frontal cortex and thalamus, arginine in the frontal cortex, glycine in the nucleus caudatus, hippocampus and mesencephalic reticular formation, and lysine in the frontal cortex and nucleus caudatus. The concentration of glutamic acid, tyrosine, serine and histidine underwent no changes in brain structures examined (Table II). The largest changes occurring in GABA showed significant decrease in nucleus caudatus and increase in the frontal cortex and mesencephalic reticular formation. The relatively short REM sleep deprivation produced alterations in the concentrations of several amino acids in various brain regions. The most frequent changes were observed in GABA and threonine concentrations. The fact that the amount of glutamic acid, as precursor of GABA in brain, remained unchanged in the structures where the amount of GABA was increased, or decreased, suggests that, inspite of the metabolic relations between those two amino acids, REM sleep deprivation may create a condition where they are independantly regulated. The involvement of GABA and glutamic acid in the control of states of vigilance has been shown by JASPER, KHAN and ELLIOT[10]. It is of interest to emphasize that 2 acids which showed significant changes in concentrations, i.e. glycine and GABA, were considered as the inhibitory transmitter agents[11]. The fall of GABA concentrations within nucleus caudatus, and aspartic acid increase in frontal cortex and hippocampus may be involved in increased neural excitability associated with REM sleep deprived state[12]. The present results could only suggest the possible amino acids participation in neurochemical mechanisms governing the states of vigilance.

Résumé. La concentration de certains acides aminés libres au niveau du cerveau chez le chat change sous l'influence de la privation élective du sommeil paradoxal. Ces changements pourraient s'expliquer par l'augmentation d'excitabilité nerveuse associée à la privation élective du sommeil paradoxal.

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Transport of Cortisol by Cultured Chronic Lymphocytic Leukemic Lymphocytes

It was reported recently that certain cultured mammalian cells have the ability to actively extrude cortisol[1]. Human lymphocytes do not concentrate cortisol above the external concentration[2], and, since these cells have specific cortisol receptors[3], the possibility that lymphocytes too, have a similar energy-dependent cortisol extrusion process, seemed worthy of investigation. Furthermore, since results from our previous studies[4,5] indicate that the presence of plasma was required in order for cortisol to have an inhibitory effect on the synthesis of lymphocyte protein, the possible influence of plasma on the uptake of cortisol was also studied.

Materials and methods. The preparation of lymphocytes suspended in autologous plasma or saline has been previously described[4-6]. 0.6 ml of lymphocytes (10^7 cells) were cultured at 4 or 37 °C in 2 ml of TC 199 medium containing 1 Ci of ^{3}H-Hydrocortisone (New England Nuclear Corp., 9 C/m*M*) for the periods of time stated in the text. At the end of each incubation period 10 ml of TC 199 medium whose temperature corresponded to that of the culture, were added and the cultures centrifuged at $1500 \times g$ for 4 min. The culture tubes were quickly inverted, drained carefully with the aid of cotton swabs, the cell button suspended in 0.5 ml of saline, the contents transferred to scintillation vials, the tubes washed 5 times with 3 ml of Brays scintillant and the washings transferred to the vials. The activities of each vial were determined with the aid of a Picker Ansitron II Liquid Scintillation Counter (efficiency of 53% for tritium) and each activity was corrected for background by subtracting the CPM of identically treated blank cultures. Quenching was found to be negligible and hence no further corrections were necessary.

Results. The uptake of cortisol by chronic lymphocytic leukemic lymphocytes (CLL) cells cultured at 4 and 37 °C

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in medium containing or lacking autologous plasma is presented in Table I. From this data 4 main observations can be made and they are: 1. Under all conditions employed, the peak of cortisol uptake occurs within 15 min. 2. A decline follows this peak, this decline is greater at 4 °C, least at 37 °C. 3. At 4 °C the peak uptake is greater than at 37 °C. 4. Total uptake is always greater in systems containing autologous plasma.

The above data suggests that some plasma factor markedly influences the uptake of cortisol. Since our previous studies indicated the importance of plasma in the cortisol mediated inhibition of lymphocyte protein synthesis, and, since it was tentatively suggested that the required plasma factor was transcortin[4,5] (the cortisol binding protein of human plasma[7]), autologous plasma was heated at 60 °C for 15 min in order to destroy the cortisol binding capacity of transcortin[8]. The results presented in Table II indicate that such heating markedly inhibits the uptake of cortisol.

Table I. The uptake of cortisol by cultured CLL lymphocytes and the effect of plasma and temperature

Time (min)	CPM at 4 °C per 10^7 cells		CPM at 37 °C per 10^7 cells	
	+ Plasma S.D.	— Plasma S.D.	+ Plasma S.D.	— Plasma S.D.
5	255 ± 25	125 ± 20	500 ± 40	335 ± 25
15	1820 ± 60	840 ± 65	760 ± 45	400 ± 30
30	400 ± 35	180 ± 30	640 ± 40	275 ± 30

Table II. The effect of heating plasma on the uptake of cortisol by 10^7 CLL cells at 37 °C

Time (min)	CPM Unheated plasma	CPM Heated plasma
15	870	200
30	634	50

Table III. The effect of $10^{-3}M$ DNP on the uptake of cortisol at 4 °C and 37 °C by 10^7 cells

Time (min)	CPM at 4 °C		CPM at 37 °C	
	— DNP	+ DNP	— DNP	+ DNP
15	440	305	385	320
30	280	300	280	280

The results presented in Table I illustrate as previously noted, that the extrusion of cortisol by CLL cells occurs after 15 min of incubation. In order to determine the dependence of this process on cellular energy, CLL cells were first incubated in medium containing DNP ($10^{-3}M$) for 15 min and then H^3-cortisol was added. The results presented in Table III indicate that both the uptake and extrusion of the hormone are inhibited by DNP.

Discussion. The results obtained in this preliminary study suggest that the uptake of cortisol by CLL cells is influenced by some plasma factor (transcortin?), dependent upon cellular energy, and is inversely related to temperature. Although the latter two observations are in agreement with those of MUNCK and BRINCK-JOHNSEN[9], and SCHAUMBURG and BOJESEN[10], the positive influence of a plasma factor on the uptake of the hormone and the extrusion of the hormone are new observations. It is interesting to note that SCHAUMBURG and BOJESEN discuss the remarkable resemblance of the thymic cortisol receptor to transcortin further supporting the possibility that the plasma factor influencing cortisol uptake and perhaps entering the lymphocytes as a complex with the hormone, is transcortin. Further studies are currently in progress whose experimental design has been pointed toward the investigation of the above relationship.

Zusammenfassung. Die Aufnahme von Cortisol in Lymphozyten wird durch ein thermolabiles Plasmaprotein begünstigt.

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Chicken Gizzard, a Myoglobin Containing Smooth Muscle

It has recently been reported that myoglobin is not detectable in human uterine muscle[1,2], and the question has been raised by FASOLD et al.[2] as to whether myoglobin is at all synthesized by smooth muscle cells. We now wish to present evidence that one type of smooth muscle, namely chicken gizzard muscle, appears to contain myoglobin or a myoglobin-like substance.

Chicken gizzard muscle has been established as a smooth muscle by both its morphology[3,4] and its biochemical properties[5,6]. Recently, in our own laboratory, immunochemical studies have shown the antigenic similarity of chicken gizzard myosin to the actomyosins of other smooth muscle fibres and its dissimilarity to the actomyosins of striated and cardiac muscle.

For the preparation of myoglobin extracts, the muscle layer of fresh or frozen chicken gizzards was quickly removed and homogenized at 4 °C with 2 volumes of water. After filtering through gauze, such extracts were centrifuged at 16,000 × *g* for 30 min and the supernatant solutions were either used directly or after lyophilisation and redissolution. When these extracts were applied to a Sephadex G-50 column (2 × 93 cm, equilibrated with 0.01 *M* phosphate buffer, pH 7.4), 2 distinctly separated reddish-brown fractions were eluted, using the same