was employed to provide light stimuli of varying durations to the pineal body. Electrical activity was monitered via a Tektronix 122 amplifier connected to a Tektronix 502 oscilloscope.

The only potentials observed having a pineal origin were sustained, low amplitude spikes with a frequency of 5–10/sec which were detected when a wick electrode was on the most basal remnant of the pineal stalk. They were not in any way influenced by changes of illumination. MORITA[1] described similar trains of impulses from ventral positions in the pigeon pineal.

The pineals of 12 English sparrows (*Passer domesticus*), 30–120 days of age, prepared according to methods 3 and 4 above, failed to show any activity relatable to changes of illumination but did with wick electrode recordings exhibit spontaneous spikes like those of the quail pineal.

There appears to be a correlation between recordable electrical activity and degree of development of presumed photoreceptors in pineal complexes. This is best supported by studies of frogs, whose pineals are electrically responsive to light[2,3] and contain recognizable photoreceptor units similar to those of the vertebrate lateral eye[4]. The evidence from light microscopy concerning the possibility of photoreceptors in bird pineal bodies is conflicting[5,6]. Electron micrographs reveal the presence of receptor-like structures, but they do not appear to be typical photoreceptors. They generally are described as rudimentary[7,8] and as lacking a well-developed outer segment[9–11]. However, the negative findings to date are an inadequate sample on which to base conclusions and do not exclude the possibility that some birds may have pineals that are photoreceptive in the conventional sense. Within other phyla there are great variations in the degrees of development of the pineal complex. This is notably true in Reptilia, some of which have highly differentiated photoreceptive units[12] as part of their pineal complex while in others a pineal may be absent[13,14].

Résumé. Dans l'épiphyse de 2 espèces d'oiseaux il n'a pas été possible de démontrer une relation entre le potentiel électrique et les variations d'illumination. On peut en conclure que chez ces oiseaux l'épiphyse n'est pas un photorécepteur dans le sens conventionnel.

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Free Amino Acids in Blood Serum of Hedgehogs in Deep Hypothermia and after Spontaneous Arousals[1]

Very little information is available concerning the relation of nitrogen metabolism to natural mammalian hibernation. In general, no 'disturbances' are considered to occur, but a quantitative reduction is evident in hibernating animals[2]. In recent years more attention has been paid to the periodicity of mammalian hibernation and many of the older data overlooking this point have had to be rechecked. With controlled experiments it appeared that the non-protein nitrogen (NPN) of the blood was lowest in animals (*Citellus lateralis*) in deepest hibernation, but a gradual increase of NPN towards the end of the hypothermia period was not observed[3]. No rise of the blood urea level could be demonstrated during the hypothermia period at the approach of spontaneous arousal or in aroused hedgehogs (*Erinaceus europaeus* L.)[4]. Changes in blood creatine levels have been demonstrated during the hibernation cycle[5].

The present report describes the blood serum free amino acid levels found in hedgehog in midwinter in deep hypothermia and after spontaneous arousal as well as in active, awake animals outside the hibernation season.

Hibernating animals were caged in a constant ambient temperature of 4 °C. The animals were transferred in this hibernaculum on October 27. No food or water was available to them. The hibernation was supervised by continuous body temperature measurements from each animal via chronically implanted thermocouples[6]. Sampling was done in midwinter after about $2^1/_2$ months hibernation. At that time the animals had undergone 15–18 undisturbed, spontaneous arousals and entries into deep hypothermia. The hypothermic group was killed after 3–4 days in deep hypothermia. Blood samples from spontaneously aroused animals with 'normal' body temperature were withdrawn 3 h after the increasing body temperature had reached 15 °C. The control group of normothermic animals was composed of animals awake

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in spring, collected from the wild, fed in the laboratory for 2 weeks and fasted for 1 day before sampling.

Blood samples were taken by heart puncture. Analyses were made from blood serum. The samples were treated by the method of STEIN and MOORE[7] and stored at − 25 °C before analysis. Analyses were run with the Beckman® 'Unichrom' amino acid analyzer[8,9]. Peaks were identified by runs with standard mixtures and by ratios calculated from absorptions at 440 and 570 nm[10].

Using this method, glutamine and asparagine emerge as a single peak. The values for glutamic acid are presented with reservation[7]. At least they may be regarded as indicative, since the treatment of samples was in every case identical. Cystine cannot be determined by this method[7].

A general survey of the results reveals that the free amino acids in the serum of hedgehogs is of about the same order of magnitude as in other mammals analysed, except that the amount of taurine is extremely high[11–14]. A comparison of groups, animals in deep hypothermia versus animals spontaneously aroused, reveals an abundant increase of free amino acids after arousal. The total amount (in μM) is more than two-fold. This may serve as an explanation of the diminished NPN of deeply hibernating animals previously reported[3].

However, this increase after the arousal process is not balanced. The arginine level was found to be decreased, whilst valine, leucine and isoleucine are not significantly increased in aroused animals, although the means are somewhat higher.

A comparison of animals that had aroused spontaneously in midwinter with animals awake in spring revealed that the total amount of free amino acids is almost equal, being only 5% less in the aroused animals. Concerning the levels of individual amino acids, some differences were observed. In the awake animals the glutamic acid was diminished and probably also methionine; arginine, glycine and probably also tyrosine on the other hand showed increased levels.

The most prominent effect of fasting on the free amino acids of plasma in normothermic animals is the increase of branched chain amino acids[12,15,16]. The hedgehogs analysed in this work had been fasting about $2^1/_2$ months in midwinter. From this point of view, if the deeply hypothermic group is compared with animals outside the hibernation season, some kind of 'fasting effect, can be considered to persist. The relative amounts of valine, leucine and isoleucine are high as compared with the other amino acids, although all are greatly decreased. On the other hand, this 'fasting effect' cannot be demonstrated between groups of aroused animals in midwinter versus animals awake outside the hibernation season.

These differences between deeply hypothermic and aroused animals are a repeated phenomenon in the hibernation cycle. It has been shown that the liver is able to regulate the levels and pattern of free amino acids in the plasma[17]. Differences in the relative amounts of free amino acids between deeply hibernating and aroused

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Free amino acids in blood serum of hedgehogs

	Hibernating in midwinter				Awake in spring
	In deep hypothermia	$P <$	Spontaneously aroused	$P <$	
Taurine	10.30 ± 4.36 (5)	0.02	29.00 ± 11.82 (4)		40.63 ± 8.13 (7)
Aspartic acid	0.58 ± 0.11 (5)	0.01	1.18 ± 0.26 (5)		1.14 ± 0.32 (7)
Threonine	3.86 ± 0.32 (5)	0.01	13.74 ± 2.74 (5)		12.67 ± 3.42 (7)
Serine	5.36 ± 0.72 (5)	0.01	13.60 ± 4.25 (5)		12.97 ± 3.62 (7)
(Asparagine + glutamine)	28.96 ± 6.23 (5)	0.01	71.44 ± 10.17 (5)		74.06 ± 10.49 (7)
Proline	1.38 ± 0.50 (5)	0.01	4.90 ± 1.97 (5)		5.26 ± 1.17 (7)
(Glutamic acid)	6.72 ± 0.71 (5)	0.01	23.46 ± 6.26 (5)	0.01	10.46 ± 2.81 (7)
Citrulline	0.82 ± 0.13 (5)	0.01	3.47 ± 0.87 (4)		4.50 ± 0.99 (7)
Glycine	7.38 ± 0.91 (5)	0.01	23.90 ± 3.45 (5)	0.02	32.03 ± 6.36 (7)
Alanine	11.72 ± 3.52 (5)	0.01	33.46 ± 5.72 (5)		33.64 ± 7.08 (7)
Valine	11.12 ± 2.73 (5)		18.64 ± 5.42 (5)		20.46 ± 2.32 (7)
Methionine	0.78 ± 0.17 (5)	0.01	3.74 ± 0.73 (5)	0.05	2.70 ± 0.80 (7)
Isoleucine	5.78 ± 1.51 (5)		7.76 ± 2.90 (5)		8.97 ± 1.08 (7)
Leucine	13.48 ± 0.49 (5)		17.82 ± 6.14 (5)		15.67 ± 1.92 (7)
Tyrosine	2.50 ± 0.49 (5)	0.01	4.00 ± 0.66 (5)	0.05	5.60 ± 1.64 (7)
Phenylalanine	4.88 ± 0.75 (5)	0.01	9.46 ± 1.76 (5)		8.57 ± 1.16 (7)
Ornithine	10.93 ± 3.33 (4)	0.02	19.32 ± 4.87 (5)		19.24 ± 6.06 (5)
Lysine	14.70 ± 1.78 (5)	0.01	24.38 ± 4.77 (5)		31.76 ± 9.20 (5)
Histidine	0.90 ± 0.25 (4)	0.01	4.56 ± 0.98 (5)		3.42 ± 1.04 (5)
Tryptophan	< 0.50		< 0.50		< 0.50
Arginine	1.57 ± 0.43 (4)	0.02	0.50 ± 0.53 (5)	0.01	3.15 ± 1.15 (4)

All values in μM/100 ml ± SD (No. of animals). P-values were obtained by Student's t-test.

animals, particularly concerning the branched chain amino acids, may depend on the distinct mediating system for the transport of amino acids[18]. Further: the 'functional' temperature of the animal in deep hypothermia is about 5 °C, whereas after spontaneous arousal, a stupendous physiologic effort, it is about 35 °C.

Zusammenfassung. Die Serumkonzentration von 21 freien Aminosäuren wurde bei Igeln im Winterschlaf, bei spontanem Erwachen während des Winterschlafs und nach dem Aufwachen im Frühling bestimmt. Mit Ausnahme von Valin, Leucin, Isoleucin und Tryptophan sinken die Aminosäuren während des Schlafes signifikant ab und steigen beim Erwachen im Winter und Frühling an.

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Structure-Activity Relationship of Various Acyl Derivatives of 6-methyl-8β-aminomethyl-10α-ergoline (Dihydrolysergamine)

The natural ergot alkaloids and their derivatives have manifold activities (adrenolytic, anti-5-hydroxytryptamine, spasmogenic, on central nervous system etc.). In general they are not very specific, for instance ergometrine, which is mainly oxytocic, causes also peripheral vasoconstriction, hyperthermia, and antagonizes 5-hydroxytryptamine (5-HT); ergotamine, which is mainly adrenolytic, and methysergide (*N*-[1-(hydroxymethyl)-propyl]-1-methyl-D-lysergamide), which is mainly anti-5-HT, share also oxytocic and vasoconstrictor activities[1-3].

The following is a brief outline of the structure-activity (oxytocic, anti-5-HT, and adrenolytic) relationship of a new series of acyl derivatives of 6-methyl-8β-aminomethyl-10α-ergoline (dihydrolysergamine)[4-9] in the attempt to find compounds with a more specific activity. The pharmacological properties of 1 of these derivatives, the acetyl-dihydrolysergamine (compound I) which has specific oxytocic activity, were further studied in comparison with ergometrine and methergine (methylergometrine).

Structure-activity relationship of carboxylic acid derivatives. The nature of the acyl residue was found to influence strongly the pharmacological activities of the parent compound (dihydrolysergamine). Compounds I, III, IV, VIII, XI and XXVI showed specific oxytocic activity comparable or superior to that of ergometrine, while compound XXVIII showed high and specific adrenolytic activity. These findings contradict the general statement that ergoline derivatives are almost inactive oxytocics.

Acylation of dihydrolysergamine yielded compounds (from I–XXII) with a prominent oxytocic activity. This activity, and the toxicity also, increased up to a certain point with the lengthening of the carboxylic aliphatic chain (I, VIII, XI). Longer chains (XIII, XIV, XV) or the substitution of the aliphatic residue with an aromatic residue (XVI, XX, XXI, XXII) caused marked reduction of the activity on the uterus. Hydroxylation in position 10 (III and XIX) or methylation in 16 (IV and XVII) did not modify (but eventually reinforced) the specific oxytocic activity already present in the parent compounds (I and XVI). When either R_1 or R_4 were not hydrogen, the derivatives (II, IX, XII, V, VI, VII, X, and XVIII) lost the oxytocic properties of their parent compound (I, VIII, XI, and XVI). Nevertheless, methylation in position 1 afforded substances (II, IX, and XII) which showed prominent anti-5-HT and some adrenolytic properties.

Structure-activity relationship of carbonic acid derivatives. When R_3 was a carboalkoxy group, the compounds (from XXIII–XXIX) were found to be pharmacologically very active (as oxytocics, adrenolytics, and anti-5-HT) but less specific and more toxic than the acyl derivatives previously discussed. Here again, the oxytocic activity disappeared when R_3 was an aliphatic chain with more than 4 carbon atoms, whereas the adrenolytic activity was not similarly affected and in one instance it became specific (compound XXVIII).

Methylation in N_{17} (R_4) yielded an inactive compound (XXV), while methylation in N_1 strengthened the anti-5-HT activity (XXIV, XXVII, and XXIX) and left almost unaltered the other properties. The introduction of a hydroxyl group in position 10 yielded a compound with specific oxytocic activity (XXVI).

All the 10β-(*cis* junction) analogues[5] of the most active compounds here described were not reported in the Table, but were also examined and found to be inactive in all our tests.

Comparison between the pharmacological actions of compound I, ergometrine, and methergine. Since compound I showed a good specific oxytocic activity and a low toxicity, further pharmacological studies were performed on it in comparison with ergometrine and methergine.

It was seen that its oxytocic activity on the uterus in situ was comparable qualitatively and quantitatively to that of ergometrine and methergine. After small doses (0.02–0.1 mg/kg i.v.) it evoked contractions in silent uterus or increased in force and frequency those already present; after progressively larger doses, it caused first forceful and tetanic contractions with increased resting tonus and then sustained contracture. The approximate

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