binary mixture of the following types: Tf M/M, Tf O/O and Tf R/R, but all other combinations are separable. One can also partially separate both bands of a phenotype by choosing an adequate NaCl gradient. The absorbancy curve of the eluted fractions shows in this case 2 shoulders on each side of a main peak. The first one corresponds to the cathodic band and the second one to the anodic band. The main peak is a mixture of both bands. In Figure 2 can be seen the elution curve of a heterozygous phenotype, Tf H/R. In this case, the curve shows 2 main peaks, the first corresponding to Tf R/R and the second to Tf H/H instead of 1 main peak as in the case of a homozygous phenotype. It is interesting to note that a third very narrow band appears at the very end of the chromatogram for each phenotype, whatever its type. This band is also visible on the pherogram of a whole serum saturated with iron. The iron-binding capacity of the compound contained in this band has been demonstrated[4]. Therefore each phenotype of the horse serum transferrins exhibits not 2 but 3 bands by starch gel electrophoresis. Some characteristics of these bands will be reported elsewhere.

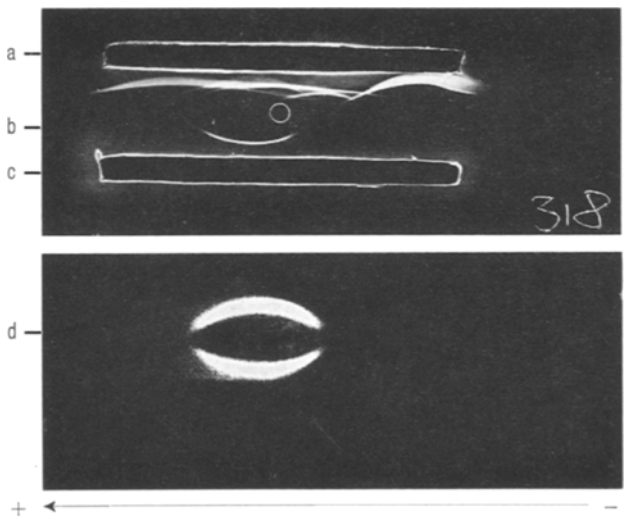

Fig. 3. Immunoelectrophoresis of a whole horse serum (micromethod of SCHEIDEGGER) and its autoradiogram as described in the text.

Immunologically, a band of purified transferrin shows only one precipitine line with an immunserum against a whole horse serum. An antiserum prepared against a purified transferrin shows likewise only 1 precipitine line with a whole serum (see *b* and *c* of Figure 3). This is illustrated by immunoelectrophoresis in Figure 3 where *b* is the circular dish containing the antigen (whole horse serum labeled with 4 μC of ^{59}Fe/ml). Dish *a* contains an immunserum against the pool of transferrins fractions mentioned above. An immunserum against pure transferrin is contained in dish *c*; *d* is an autoradiogram of the same experiment. The circular dish is barely visible but the position of the inferior line is exactly the same as that of the line between *b* and *c*, thus demonstrating that the latter corresponds to the transferrin.

One can obtain a larger amount of almost pure transferrins by precipitating the serum, diluted with 1 volume of physiological NaCl and adding enough ammonium sulfate to bring the final concentration to 56% in ammonium sulfate. This precipitation is substituted for the gel filtration and ion-exchange chromatography is replaced by a batch operation. The transferrins thus obtained are still contaminated by 2 albumins which can be eliminated by column chromatography[5,6].

Résumé. Les bandes des différents phénotypes de transferrines (Tf), identifiées par amido-électrophorèse, ont été purifiées par filtration sur gel et chromatographie échangeuse d'ions. Des anticorps de lapin ont été préparés contre plusieurs de ces phénotypes.

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Influence of Season and Activity on Sodium Content of Bones and Plasma of the Bat, *Myotis lucifugus*

A number of workers have noted changes in plasma levels of certain minerals during the hibernal siege, but there are few reports concerning fluctuations in bone levels of these elements. Furthermore, no attempt has been made to determine what effects simple inactivity might have upon hibernators' skeletons if the animals were not hibernating. This study was part of an attempt to clarify the status of major minerals in a hibernator, *Myotis lucifugus*, during natural hibernation and in 2 activity states while kept under laboratory conditions.

Methods. Hibernating bats were captured in a southern Indiana cave at 3 stages of the hibernating season (early, November; deep, February; late, April). In the laboratory they were housed in a moist, cold (8 ± 2 °C), darkened room until sacrifice shortly after capture. Summer bats were captured in June from an attic colony and sacrificed immediately. Bats designated 'winter free-flight' and 'winter restricted' were procured from a colony in early fall. The 'free-flight' bats were weighed, sexed, coded, and placed into a screened cage of 450 ft^3 flying space. The cage was maintained in a room at 35 °C with a relative humidity of 20%. The 'winter restricted' bats were housed in the same room, but were restricted in movement by placing them in a confined space of 100 in^3. Animals designated as 'summer free-flight' and 'summer restricted' were obtained from a colony in June and housed in the same manner as the 'winter free-flight' and 'winter restricted' groups, respectively. All bats were fed mealworm (*Tenebrio molitor*) larvae, and water was provided ad libitum.

After a chronic period in the free-flight room or restraining cage, the bats were sacrificed by decapitation and blood and bone samples were retained for analysis.

Plasma sample preparation. Following decapitation, the bat was exsanguinated and the whole blood was promptly drawn into calibrated (volume = $\pi r^2 h$) microhematocrit capillary tubes[1]. Following centrifugation, the plasma supernatant was diluted to put the sodium concentration within the working range of an atomic absorption spectrophotometer (Perkin-Elmer model 303)[2]. A working curve for sodium was plotted by ordinating absorbance with known concentrations of sodium. The concentration of sodium in plasma samples was read from the curve and expressed as mEq/l.

Bone sample preparation. The bones supporting the wing membranes of the bat were analysed for sodium content. After removal of extraneous tissue, the bones were weighed, dried, and ashed in a muffle furnace. Bone ash was dissolved in concentrated HCl and appropriately diluted. The concentration of sodium in bone was determined as for plasma, and expressed as mEq/kg.

Results. The concentration of sodium in bat plasma and bone under varying natural and laboratory conditions is presented in the Table. An inspection of these data reveals that the sodium concentration of bone decreases as hibernation progresses, and begins to return to higher levels in summer bats. Bone sodium is greater in both winter and summer free-flight animals than in their restricted counterparts. A consistent pattern of sodium changes in plasma is not evident in the hibernating animals, although sodium concentration is greatest in plasma of bats which have just entered hibernation. The plasma picture is quite consistent in showing higher concentrations of sodium in the restricted bats as compared with the free-flight bats, in both winter and summer. Some of the differences observed are statistically significant, as indicated by Student's *t*-test (Table).

Discussion. Previous studies have shown that chronic immobility of a limb or the entire body precipitates mineral loss from the skeleton. The results of this study affirm that this occurs during hibernation in the little brown bat, for bone sodium concentration is progressively lower at successive stages of hibernation. These results agree with in vitro evidence of others[3] indicating that a lack of mechanical stress on bones leads to bone mineral loss, and support the contention that disuse osteoporosis can occur in the absence of mechanical strain[4]. Furthermore, these findings support conclusions from dental tissue studies with hibernating ground squirrels: as hibernation proceeds, the mineral drain within the animal body increases[5–7].

Diet as well as immobility undoubtedly influences sodium loss from bone, although there are indications here that the effect of diet on bone demineralization is overridden by that of immobility. Bone levels of sodium are lower in winter restricted bats than in winter free-flight bats, although both groups received identical diets. The same is true for summer free-flight and restricted bats, and the difference is statistically significant at an α-level of 0.01 (Table). The plasma sodium levels seem to be higher in early stages of hibernation, falling to half that concentration for the remainder of the hibernal period and in summer. Bone sodium is also apparently mobilized more when the bat is restricted in movement, as indicated by lower bone sodium levels in restricted than in free-flight bats in both winter and summer. Plasma values in the winter free-flight versus restricted bats support this contention, whereas plasma sodium levels in the summer laboratory animals are about the same in the 2 groups. It should be noted that the plasma sodium concentration is considerably higher in the bat on a general basis than in most mammals, according to the results of this investigation.

It seems certain that bone sodium concentrations are depleted during hibernation and during enforced immobility in winter and summer bats. The mechanism causing this demineralization is unknown, although the osteoclast under the influence of parathormone and/or other hormonal agents has been implicated[8–10].

Zusammenfassung. Plasma und Knochensubstanz von Mikrochiropteren wurden auf Natriumgehalt untersucht. Der Knochen-Natriumgehalt nimmt im Verlauf der Winterschlaflethargie ab und kehrt zu höheren Werten bei Sommerfledermäusen zurück. Die Knochen-Natriummenge ist bei aktiven Fledermäusen, die in ihren Bewegungen während des Sommers und Winters behindert wurden, niedriger als bei freigelassenen Fledermäusen. Dies zeigt, dass die Bewegung keinen starken Einfluss auf den Mineralgehalt der Knochen hat. Der Plasma-Natriumgehalt der Fledermäuse ist höher als in den meisten andern Säugetieren und scheint zudem zu Beginn des Winterschlafs besonders hoch zu sein.

Sodium concentration in Plasma and bone of *Myotis lucifugus*

Treatment	Plasma (mEq/l) $\bar{X}$	S.D.	*t*-value	Bone (mEq/kg) $\bar{X}$	S.D.	*t*-value
Early hibernation	637	± 108	7.353[a]	1162	± 73	11.614[b]
Deep hibernation	347	± 68	0.900	713	± 107	0.164
Late hibernation	371	± 48	0.481	705	± 108	2.112[c]
Summer	385	± 77		793	± 73	
Winter free-flight	377	± 83	0.438	786	± 92	0.431
Winter restricted	419	± 157		772	± 41	
Summer free-flight	296	± 39	0.121	609	± 67	3.309[b]
Summer restricted	259	± 52		513	± 62	

[a] Significant at the 0.01 level: $t_{0.01}$ (20) = 2.845.
[b] Significant at the 0.01 level: $t_{0.01}$ (18) = 2.878.
[c] Significant at the 0.05 level: $t_{0.05}$ (18) = 2.101

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