

PDB-1 Chicago. Three specimens were unaltered and gave reproducible results. The data are as follows:

1. Most negative specimen: Sample 1: $\delta = -2.59\text{‰}$
Sample 2: $\delta = -2.54\text{‰}$
Sample 3: $\delta = -2.52\text{‰}$
Average: $\delta = -2.55\text{‰}$
2. Sample 1: $\delta = -2.30\text{‰}$
Sample 2: $\delta = -2.30\text{‰}$
Sample 3: $\delta = -2.19\text{‰}$
Average: $\delta = -2.26\text{‰}$
3. Sample 1: $\delta = -1.75\text{‰}$
Sample 2: $\delta = -1.70\text{‰}$
Sample 3: $\delta = -1.65\text{‰}$
Average: $\delta = -1.70\text{‰}$

Assuming the mean ocean δ to have been zero (as at present), the paleotemperatures corresponding to the average values listed are as follows:

- Specimen 1: 28.4°C
Specimen 2: 27.0°C
Specimen 3: 24.2°C

The significance of these results is apparent when they are compared with others obtained by the author elsewhere in Europe³. From the Toarcian (upper Lias) of Yorkshire and Northampton temperatures of 31.7°C and 29.6°C, 25.7°C, 23.8°C respectively were obtained. A specimen of the same age from Thouars in France gave a temperature of 24.9°C, while two other specimens from the Pliensbachian of St. Vincent Herlanges gave temperatures of 24.6°C and 24.3°C respectively. Measurements were made on three Toarcian Belemnoida from Switzerland. One, from Sulz (Fricktal, Kanton Aargau), gave a temperature of 27.3°C. A second, from Les Pueys (Canton de Fribourg), gave 23.5°C. The third, from Le Taboussset (Canton de Vaud), gave 23.4°C. The data show a considerable uniformity of temperature to have existed throughout Western Europe in Liassic times. They also show that this was a high temperature and this can be demonstrated too by comparing them with temperatures recorded from, e.g., the Middle Jurassic of France (range: 19.5°C to 21.8°C) and the Upper Jurassic of Germany (range: 20.5°C to 21.8°C)³. The Toarcian and Pliensbachian emerge as very warm, equable time intervals. The scarcity of limestones and coral reefs at this time, however, has led

some workers⁴ to infer cooler conditions than in the later Jurassic. That this conclusion is erroneous is demonstrated empirically by the oxygen isotope analyses, but it can be shown to be mistaken on other grounds. The formation of limestone depends on temperature and also on the amount of inorganic clastics being introduced into the area of potential limestone formation. Thus limestone, even under optimum temperature conditions, may not form because of excessive dilution by influx of non-calcareous sediment⁵. An interesting point in this connection is that at least in one case, a Caribbean core, carbonate content and temperature are unrelated⁶. In addition, it is worth mentioning the rich flora of temperate facies found in the early Lias of East Greenland⁷. The universal distribution of Ammonoid faunas in the Lower Jurassic leads to the inference that the high temperatures found so uniformly in the upper Lias probably existed throughout the rest of the Lias as well. Further analyses are necessary to demonstrate this physico-chemically.

Zusammenfassung. Belemniten aus dem Lias epsilon von Trimeusel bei Bamberg (Bayern) wurden mit Hilfe von Sauerstoffisotopen analysiert und die Palaeotemperatur bestimmt. Vergleiche mit Belemniten aus dem unteren Lias diverser europäischer Gebiete ergaben den Nachweis einer Periode mit warmen Meerwasser. Dies steht im Gegensatz zu Angaben anderer Autoren, die auf dem Fehlen von Kalkablagerungen und Korallenriffen basierten.

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R. BOWEN, *J. Geol.* 69, 309 (1961).

⁴ M. SCHWARZBACH, in *Descriptive Palaeoclimatology* (Ed. A. E. M. NAIRN, Interscience Publishers Inc., New York 1961), p. 255.

⁵ G. Y. CRAIG, in *Descriptive Palaeoclimatology* (Ed. A. E. M. NAIRN, Interscience Publishers Inc., New York 1961), p. 207.

⁶ R. YALOVSKY, *J. Geol.* 65, 480 (1957).

⁷ W. J. ARKELL, *Jurassic Geology of the World* (Oliver and Boyd Ltd., Edinburgh and London 1956).

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Structure of the Nephridia of the Indian Cattle Leech *Hirudinaria granulosa* (Savigny) with Remarks on their Nephridial Microflora

BHATIA¹ confused the bacteria present in the nephridial bladders of *Hirudinaria granulosa* with cilia and described their size, distribution, and vibratility. In fact the fluid, filling the nephridial bladders, was turbid in appearance due to excessive bacterial growth in it. The bacilli were mostly non-motile (vibratility being due to the Brownian movement), 2.8 to 7 μ (average 4 to 5 μ) in length, Gram-negative, non-acid fast and formed macroscopic clumps. The vesicles were internally lined with a whitish layer of the same type of bacilli, which could be removed by means of a brush. The layer was thickest in the upper part of the vesicle and its thickness diminished towards the nephridiopore. On the postero-dorsal side of each vesicle was present a whitish patch, formed of a comparatively better growth

of bacteria, coinciding with the opening of the vesicle-duct into the nephridial bladder. Sections of the vesicles, stained with Gram's technique^{2,3} using Hucker-Conn crystal violet, Weigert's iodine, and Saffranin, showed an uneven internal layer of Gram-negative bacilli.

The bacteria were counted by using the haemocytometer. The fluid was diluted so as to contain about 10^7 to 10^8 cells per ml for accurate counting, thoroughly churned in order to break the bacterial clumps, filled in the counting chamber and counting done under dark-ground illumination. The fluid contained 6.5 to 10.6 million bacterial cells per cm^3 (average 8.1 million).

¹ M. L. BHATIA, *Quart. J. Micr. Sci.* 81, 27 (1940).

² R. D. LILLIE, *Histopathologic Technique and Practical Histochemistry* (Blakiston Co., New York 1954), p. 501.

³ R. CRUICKSHANK, *Mackie and McCartney's Handbook of Bacteriology* (E. & S. Livinstone Ltd., Edinburgh 1960), p. 980.

The fluid, collected from the nephridial bladders under aseptical conditions, was used for plating and attempts were made to grow the bacteria on Bacto nutrient agar plates, ordinary nutrient agar plates, simple blue plates containing bromothymol blue, blood agar plates (blood of both sheep and cattle used), Loeffler's serum medium, Dorset's egg medium, Aronson's medium, alkaline peptone water, papain digest broth, cooked meat medium, Christenson's medium, aerobically, anaerobically and also in an atmosphere rich in carbon dioxide at 20°C to 37°C. Alteration in pH, temperature and even the media made with Seitz-filtered leech extract with an addition of some of the common growth factors did not promote the growth of these bacteria.

For studying the chemical characteristics of the bacteria, the fluid was collected and divided into two parts. One part was incubated at 37°C for 12 h. In the other, the bacteria were killed by ultraviolet irradiation, autoclaving or addition of toluene and then the containers were incubated at 37°C for 12 h. Both the parts were analysed after the incubation for ammonia nitrogen and urea nitrogen by Conway's microdiffusion technique⁴. Total amount of ammonia nitrogen and urea nitrogen was almost constant in the two cases. Quantity of ammonia nitrogen, however, was significantly higher and of urea nitrogen lower in the untreated fluid as compared with the respective values of the other in which the bacteria were killed before incubation. This appears to indicate that the bacteria are capable of breaking urea into ammonia. The bacteria seem to differ from the genus *Corynebacterium*, two species (*C. vesiculare* and *C. hirudinis*) of which have been reported by BÜSING et al.^{5,6} from the nephridial bladders of *Hirudo medicinalis*.

Deproteinized vesicular fluid, analysed chromatographically, contained approximately 50 to 60 µg of amino

acid nitrogen per ml. The supernatant fluid of the centrifugated vesicular fluid gave a positive reaction for proteins (albumin and globulin). The amino acids and proteins, which presumably leak out during the process of excretion, provide a rich substrate and promote the floccose growth of bacteria in the nephridial bladders. The growth is richest at the point from where the fresh excretory fluid, containing a sizeable quota of amino acids and proteins, trickles into the nephridial bladder. A thick felt-like covering on the inner wall of the bladder, and the macroscopic clumps are formed to avoid the escape of the bacteria through the nephridiopores.

Zusammenfassung. Die Nephridia von *Hirudinaria granulosa* weisen an ihrer inneren Oberfläche keine Cilien, wie es von BHATIA angenommen wurde, sondern Bakterien auf, die makroskopisch sichtbare Klumpen bilden. Ihr Wachstum wird durch grosse Quantitäten von Aminosäuren und Eiweisskörpern des Urins gefördert. Die Bakterien zeigten einen Unterschied gegenüber der Spezies der Corynebakterien. Sie konnten auf verschiedenen Nährböden nicht gezüchtet werden und spalteten Urea in Ammoniak.

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Department of Zoology, University, Lucknow (India),
March 7, 1963.

⁴ E. J. CONWAY, *Microdiffusion Analysis and Volumetric Error* (Crosby Lockwood & Son, London 1957), p. 465.

⁵ K. H. BÜSING, W. DÖLL, and K. FREYTAG, *Arch. Mikrobiol.* **19**, 52 (1953).

⁶ K. H. MANN, *Leeches (Hirudinea)—Their Structure, Physiology, Ecology, and Embryology* (Pergamon Press, Oxford 1962), p. 201.

The Fatty Acids of the Deposit Lipids in the Hibernant

It is known that hibernating animals form lipid deposits during the summer months, to supply the requirements of basal metabolism, which are notably reduced in lethargy^{1,2}.

The biochemical and physiological modifications that occur during hibernation were studied by various authors, but today many points still need further clarification.

With regard to the fatty acids, LYMAN³ has observed an increase in the iodine number during hibernation. An evaluation of the spectrum of fatty acids and of their possible variations is the object of the present research.

The investigation was conducted on the lipid deposits of *Rinolophus ferrum equinum* at three different periods during hibernation: immediately before (October), after some time (January) and at the end (June).

The methyl esters of the fatty acids have been analysed by means of a gas chromatograph with ionization chamber using a celite column with PGA as stationary phase. The temperature was 181°C and the carrier gas was argon with a rate of flow of 50 ml/min.

The results obtained from the assays of 3-4 pools of two individuals for each period are in agreement: mean values are reported in the Table and show that the saturated fatty acids do not vary appreciably, the monounsaturated fatty acids are in greater amount before and at the end of

Spectrum of the fatty acids in the subcutaneous adipose tissue of the bat (*Rinolophus ferrum equinum*) in different periods of hibernation

Fatty acids*	Before hibernation	At the beginning of hibernation	At the end of hibernation
Myristic	0.25	1.38	0.28
Palmitic	19.30	17.46	21.94
Palmitoleic	8.37	5.95	9.44
Stearic	1.09	3.42	1.03
Oleic	59.37	48.75	53.24
Linoleic	5.64	8.82	5.63
Linolenic	5.85	11.33	8.41
Saturated	20.64	22.26	23.25
Monounsaturated	67.74	54.70	62.68
Polysaturated	11.49	20.15	14.04
Ratio: 16:0/16:1	2.30	2.93	2.32
18:0/18:1	0.18	0.70	0.19

* Fatty acids are expressed in % of chromatographed fatty acids.

¹ F. G. BENEDICT and R. C. LEE, *Hibernation and Marmot Physiology* (Carnegie Inst. Wash. Publ., 1938), n. 497.

² C. KAYSER, Thesis, Fac. Sci. Strasbourg (1949).

³ C. P. J. LYMAN, *Exper. Zool.* **109**, 55 (1958).