Nucleated cells of the peripheral blood of *Stenella styx* Gray and *Delphinus delphis* L. from the western Mediterranean and description of the haemomyelogram of *Delphinus delphis*[1]

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Several papers have been published during the last ten years containing haematological and haematochemical data on various species of cetaceans obtained from aquaria or captured while at liberty (1, 9, 10, 11, 13, 14, 15).

During a recent expedition organized by Prof. R. G. Busnel in the western Mediterranean (15. VIII to 18. IX, 1966), G. Pilleri arranged for anatomical and anatomopathological material to be collected as well as blood samples and marrow smears, for use in haematological and haematochemical research on the cetaceans captured.

The present paper contains data on nucleated cells circulating in the bloodstream and a description of the haemomyelogram of a species, *Delphinus delphis* L., which until now was not been investigated. In subsequent papers we shall describe haematochemical data, also referring them to anatomopathological research, on individual specimens of the same species and of a single specimen of a species captured during Prof. Busnel's expedition defined by Dr. F. C. Fraser of the British Museum, London as *Stenella styx* Gray. With regard to the latter species, references in the present paper will be confined to observations pertaining to leukocytes in the circulation, indicated by way of comparison with *Delphinus delphis*.

Material and Methodology

For the present research, we used 8 adult specimens (5 ♂♂ and 3 ♀♀) of *Delphinus delphis* and 1 specimen ♂ of *Stenella styx* captured in the western Mediterranean in the months of August and September 1966. Most of the specimens were harpooned; only two (Nos. 369 and 377) were immobilized by electric anaesthesia. For the final enumeration of the individual specimens, the progressive numbers of the collection of the Brain Anatomy Institute of the University of Bern were used.

The blood samples for haematological research were taken from the rete mirabile surrounding the eyeball immediately after the death of the animal, and crystals of EDTA-sodium salt added in the test tube as anti-coagulant: this part of the blood was used for the assessment of the blood cells, the haemoglobin study and a few plasma investigations. Samples No. 369 and 377 were taken from the artery of the tail.

The blood smears were made in loco; some were fixed with methyl alcohol shortly

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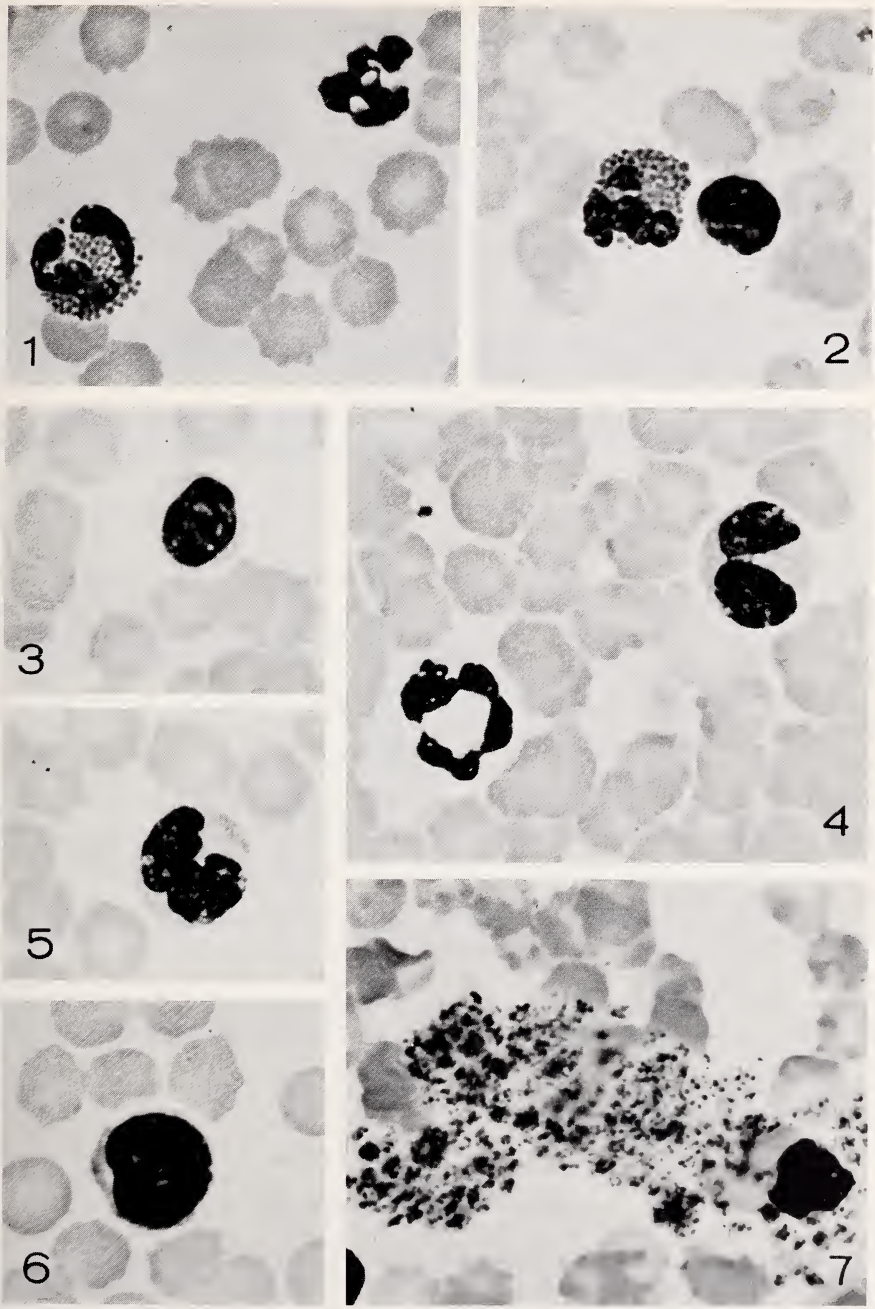

Fig. 1. Leukocytes in the peripheral blood of *Stenella styx* Gray: *1.* Neutrophilic granulocyte and eosinophilic granulocyte. — *2.* Eosinophilic granulocyte and lymphocyte. — *3.* Lymphocyte. — *4.* Neutrophilic granulocyte and monocyte. — *5.—6.* Monocytes. — *7.* Accumulation of platelets between two lymphocytes

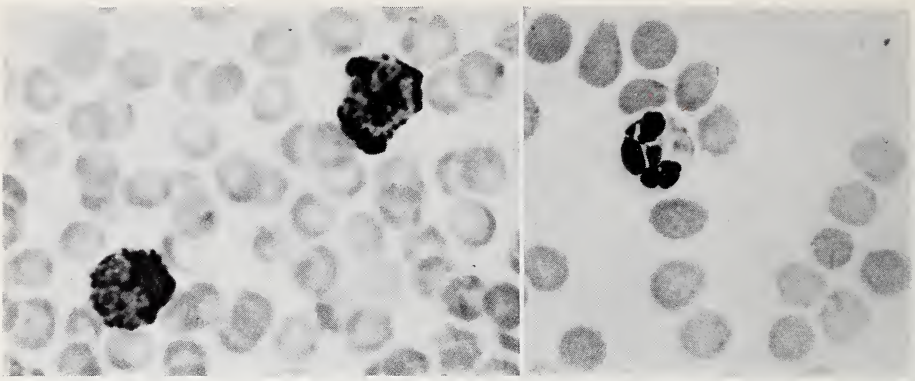

Fig. 2. Detection of "highly persistent" peroxydasis by the benzidine test (modification II of Undritz) in the peripheral blood of *Delphinus delphis* L. *Left* two eosinophilic granulocytes, *right* a neutrophilic granulocyte

after they had been taken and others were kept without fixation for subsequent peroxydasis tests.

The marrow smears were obtained from marrow taken from the scapula or top of the humerus, split into two with a knife and the sectioned surface was rubbed over a slide. It should be noted that both the scapula and the humerus of the *Delphinus delphis* present a compact structure and contain very little bone marrow.

The haematological material was investigated in Trieste, where it was flown as soon as possible after the samples had been taken.

The leukocyte counts were carried out, whenever possible, in the Bürker chamber using Türk's liquid as diluent.

For basic staining we used the panoptic method of Pappenheim with a buffer diluent of phosphates (pH = 7.2); to detect granulations with peroxydasis we used the Graham-Knoll reaction, and to bring to light the eosinophils, modification II according to Undritz (4).

The differential formula for the peripheral blood was obtained by counting an average of 400 nucleated cells for each smear.

The haemomyelogram was compiled by studying 3000 nucleated cells from three different smears of the same marrow. For the identification of the cells we prescinded from haematochemical methods and based our identification on the terminology used by Fieschi (2).

The observations were made with a Leitz microscope (1200 ×) and the microphotographs were magnified 1250 ×[2].

Results and Discussion

Results pertaining to leukocytes found in the bloodstream are set out in Table 1.

As can be seen, the total number of leukocytes per mm^3 is relatively uniform. This result should not, however, be considered reproductible as it based on blood tests carried out after a certain lapse of time (6 days or more!), but this average, that is relatively homogeneous in comparison to data obtained by other authors (1, 6, 7, 13) during

[2] The authors wish to acknowledge the helpful technical assistance of Miss M. Antonelli in the present work.

extemporaneous tests on ceteceans, could reasonably be supposed to represent a result that is at least indicative.

The neutrophilic granulocytes exhibit a noticeable variability in percentage (34.5 % to 67.25 %), the causes of which might perhaps find some explanation in the correlation between the said variability and the anatomopathological findings during the working out of results.

Basophilic granulocytes are rarely found in the circulation; we only observed one in the 400 nucleated cells investigated for each smear.

Eosinophilic granulocytes, although present on an average in a high percentage, also exhibit noticeable individual variability that is perhaps in no way related to the common and frequent parasitosis of the animal. The high percentage of eosinophilic granulocytes in the bloodstream has been repeatedly observed by all those who have

Table 1

Leukocytes in the peripheral blood of *Stenella styx* Gray and *Delphinus delphis* L.

No.-Sex-Species	Leukocytes mm³	N ·/.	B ·/.	E ·/.	L ·/.	M ·/.	Other cells ·/.	
370 ♂ *Stenella styx* Gray	7,200	52.0	0.0	10.0	33.0	5.0	0.0	
369 ♂ *Delphinus delphis* L.	7,000	67.25	0.25	1.0	29.0	2.5	0.0	
371 ♀	—	51.0	0.0	2.0	44.0	3.0	0.0	
372 ♂	8,100	44.0	0.0	26.0	26.0	4.0	0.0	
373 ♀	—	37.0	0.0	15.0	45.0	3.0	0.0	
374 ♂	8,800	57.5	0.0	10.0	30.5	1.5	0.5	Ebl.
377 ♂	6,900	48.0	0.0	9.5	40.0	2.5	0.0	
379 ♀	—	39.5	0.0	19.5	38.0	3.0	0.0	
382 ♂	—	34.5	0.0	25.0	39.3	1.0	0.2	McN
Mean values	7,700	47.35	0.03	13.5	36.48	2.56	0.08	

N = neutrophilic granulocytes, B = basophilic granulocytes, E = eosinophilic granulocytes, L = lymphocytes, M = monocytes, McN = neutrophilic myelocytes, Ebl. = orthochromic erythroblasts

studied the haematology of cetaceans (Table 2) and, in analogy to human pathology, hypotheses have been formed as to causes ascribable to parasitosis; our opinion on the possible causes of such a high average percentage of eosinophilic granulocytes in *Delphinus* and in *Stenella* can be stated very briefly. In the light of our previous experience, we are in a position to affirm that in other animal groups (*Rattus norvegicus* Berk.), even an advanced degree of infestation with Helminth worms causes no appreciable increase in the eosinophilic granulocytes in the peripheral blood.

It has, however, been determined that it is only in the Fam. Phocidae (Table 2) that the average percentage of eosinophilic granulocytes is similar to that observed in human beings: in the various species of cetaceans so far submitted to haematological studies, an almost constant increase in the percentage of eosinophils has been observed. Lower individual variability can perhaps be claimed for lymphocyte (22—44 %) and monocyte (1—5 %) percentages.

We, like other authors (6, 7, 9) have discovered in some specimens immature cells belonging either to the red series (orthochromic erythroblasts) or the white series (neutrophilic myelocytes). These are, of course, incidental and likely hypotheses attribute them to the often long survival of harpooned, bleeding specimens in water.

Table 2

Percentage composition of nucleated cells found in the blood stream of cetaceans examined up to now and of some carnivores

	Neutrophils	Basophils	Eosinophils	Lymphocytes	Monocytes	Other cells	Authors
Cetacea							
Inia geoffroiensis Blainville	34.0	0.5	22.5	38.0	4.5	—	Knoll, 1932
Stenella styx Gray	52.0	—	10.0	33.0	5.0	—	De Monte and Pilleri
Delphinus delphis L.	47.35	0.03	13.50	36.48	2.56	0.08	De Monte and Pilleri
Tursiops truncatus Montagu	15.6	0.6	11.8	38.3	17.0	16.2	Morimoto and coll., 1921
Tursiops truncatus Montagu	45—85	—	9—40	8—22	0—1	—	Medway and Geraci, 1964
Tursiops truncatus Montagu	45—75	0—1	5—30	10—30	1—6	—	Ridgway, 1965
Lagenorhynchus obliquidens Gill	33.0	—	35.0	24.0	2.0	—	Miller and Ridgway, 1963
Lagenorhynchus obliquidens Gill	40—70	0—1	5—30	10—30	1—6	—	Ridgway, 1965
Orcinus orca L.	54.0	—	1.0	38.0	4.0	3.0	Newman and McGeer, 1966
Globicephala melaena Traill	$71{,}4 \pm 18{,}7$	< 1	$7 \pm 9{,}2$	$18{,}8 \pm 6{,}2$	$2{,}6 \pm 1{,}5$	—	Medway and Moldovan, 1966
Globicephala scammoni Cope	50—70	0—1	2—20	20—40	4—10	—	Ridgway, 1965
Phocaena phocaena L.	16.0	0.5	13.0	39.0	17.0	14.5	Morimoto and coll., 1921
Phocaena phocaena L.	40—75	0—1	5—20	20—50	0—3	—	Andersen, 1965
Phocoenoides dalli True	60—85	0—1	2—5	10—20	4—10	—	Ridgway, 1965
Delphinapterus leucas Pallas	39.1	1.2	9.0	39.3	11.4	—	Quay, 1954
Physeter catodon L.	26.5	1.5	15.0	33.0	2.0	21.5	Knoll, 1932
Megaptera novaeangliae Borowski	27.0	—	22.0	50.0	3.0	5.0	Knoll, 1932
Balaenoptera physalus L.	34.0	—	25.0	33.0	2.0	6.0	Knoll, 1932
Balaenoptera musculus L.	29.0	—	17.0	48.0	4.0	2.0	Knoll, 1932
Carnivora							
Phoca vitulina L.	46—77	2—6	2—11	8—34	1—7	—	Kraft, 1962
Zalopus caliphornianus Lesson	50—70	0—1	0—3	30—40	3—6	—	Ridgway, 1965

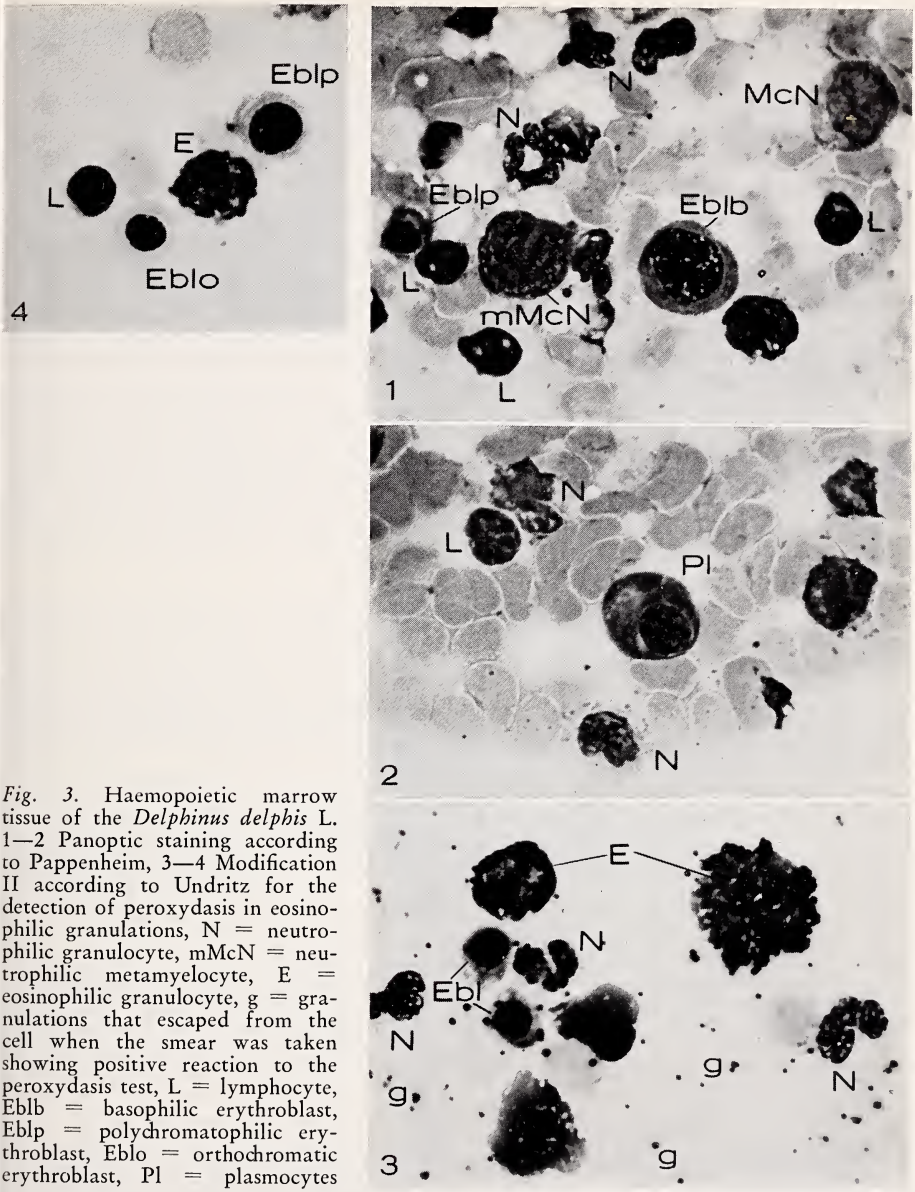

Fig. 3. Haemopoietic marrow tissue of the *Delphinus delphis* L. 1—2 Panoptic staining according to Pappenheim, 3—4 Modification II according to Undritz for the detection of peroxydasis in eosinophilic granulations, N = neutrophilic granulocyte, mMcN = neutrophilic metamyelocyte, E = eosinophilic granulocyte, g = granulations that escaped from the cell when the smear was taken showing positive reaction to the peroxydasis test, L = lymphocyte, Eblb = basophilic erythroblast, Eblp = polychromatophilic erythroblast, Eblo = orthochromatic erythroblast, Pl = plasmocytes

Data obtained up till now on the percentage composition of nucleated cells in the peripheral blood of cetaceans are set out in systematic order according to Hershkovitz (5) in Table 2.

These are results obtained by somewhat different methods with specimens that were nearly always too scarce to give a valid average. A comparison of the percentages obtained by Morimoto et al. in 1921, by Medway and Geraci in 1964, and by Ridgway in 1965, for nucleated cells found in *Tursiops truncatus* Montagu will illustrate the point: it is obvious that the findings of Morimoto and colleagues were based on the study of specimens (if not of one specimen) that had been extremely badly handled,

with the result that the values cited are in obvious disagreement with values given by other authors, and the discovery of anomalous nucleated shapes, probably ascribable to erythroblasts, lends added reliability to the aforesaid hypothesis. The same striking disagreement between the findings of Morimoto et al. and the values published by Andersen in 1965 is to be found in results for *Phocaena phocaena* L.: here, again, the values put forward by Morimoto et al. differ considerably from values normally given for the species.

The calculation of our average percentages for *Delphinus delphis* is based on results obtained from 8 specimens. This does not, however, mean that they are to be considered indicative: we deem it essential to refer the values of the differential formula of each animal to the results of the anatomopathological test. In our opinion, this is the only way to try interpret certain noticeable variations in percentages observed in single specimens and to establish, by subsequent observations, the true "normality" for animals living in their natural surroundings. It is a known fact that in aquaria cetaceans suffer comparatively frequently from a series of diseases often leading to their death: the most frequent diseases are mycosis and parasitosis that thrive in narrow surroundings and on incorrect diets. Research aimed at establishing „normal" haematological and haematochemical values for animals kept in aquaria always differ, even if only slightly, from that carried out on animals in their natural surroundings. The former is, however, of considerable value from an experimental standpoint, in that it elucidates many of the variations observed in nature.

The results of the haemomyelogram of *Delphinus delphis* L.: taken from specimen

Table 3

Haemomyelogram of *Delphinus delphis* L., ♂, No. 328 and differential leukocyte count of the same specimen compared with the haemomyelogram of a normal adult male human

	Haemomyelogram of the *Delphinus delphis*		Leukocytes in the circulation	Haemomyelogram of a normal adult human of the male sex (acc. Fieschi)
	cells examined	·/.	·/.	
Haemocytoblasts	9	0.30		0.24
Myeloblasts	91	3.03		1.52
Promyelocytes	92	3.06		2.98
Myelocytes N	135	4.50	0.20	14.70
Myelocytes E	146	4.87		
Metamyelocytes N	62	2.06		9.76
Metamyelocytes E	173	5.76		
Granulocytes N, non-segmented	201	6.70		13.20
Granulocytes N, segmented	556	18.54	34.5	18.60
Granulocytes E	528	17.60	25.0	2.40
Granulocytes B	0	0	0	0.74
Proerythroblasts and basophilic erythroblasts	102	3.40		3.56
Polychromatophilic erythroblasts	328	10.94		11.56
Orthochromatic erythroblasts	151	5.03		9.40
Lymphocytes	233	7.44	39.3	4.18
Monocytes	42	1.40	1.0	2.44
Histiocytes	30	1.00		1.76
Plasmocytes	11	0.37		0.96
Megacaryocytes	0	0		0
Non-identifiable cells	120	4.00		2.00

♂No. 382, are set out in Table 3; the percentage of leukocytes found in the circulation of the same specimen and percentages for a normal adult human male are also indicated.

A striking feature is the remarkable similarity between the percentages of the two haemomyelograms, with the exception of the noticeable increase in the eosinophil series that reflects the high percentage of granulocytes present in the peripheral blood of *Delphinus*. Because of the large number of eosinophils present (28.23 % of the marrow elements), we consider it almost essential to carry out, at least on one marrow smear, a test for peroxydasis granules by modification II according to Undritz (4), aimed specificially at detecting the "highly persistent" peroxydasis characteristic of eosinophil granulations. The difficulties encountered in discriminating between the various stages of maturing in both the white and erythrocyte series were no greater than those met when compiling the human haemomyelogram: as always the preparation of the smears and the time taken to stain them play an extremely important role.

At the moment we are not in a position to offer adequate comments on the facts set out in the haemomyelogram, as we lack comparative data from other cetaceans. We hope, however, in the near future, to be able to carry out the same test on other species closely or less closely related to the *Delphinus*.

Summary

Percentage values for nucleated cells circulating in the peripheral blood of *Delphinus delphis* L. and *Stenella styx* Gray have been given and these have been compared with values published in literature for other cetaceans. The paper also contains the first description of the haemomyelogram of a cetacean, *Delphinus delphis* L.

Zusammenfassung

Die Prozentwerte der kernhaltigen Zellen im peripheren Blut von *Delphinus delphis* L. und *Stenella styx* Gray werden mitgeteilt und mit den Befunden der Literatur über andere Cetaceenarten verglichen. Zum ersten Mal wird das Haemomyelogramm einer Cetaceenart, *Delphinus delphis*, beschrieben.

Riassunto

Vengono resi noti i valori percentuali relativi alle cellule nucleate circolanti nel sangue periferico di *Delphinus delphis* L. e di *Stenella styx* Gray e questi reperti vengono confrontati con quelli rinvenuti nella letteratura per altri cetacei. Inoltre per la prima volta viene descritto l'emomielogramma di un cetaceo, il *Delphinus delphis*.

Resumé

Les valeurs en pourcents des cellules nuclées dans le sang périphérique de *Delphinus delphis* L. et de *Stenella styx* Gray sont communiqués et comparées ensuite aux données publiées jusqu'ici sur d'autres espèces de Cétacés. Dans le prèsent travail, le hémomyélogramme d'une espèce de Cétacés, *Dephinus delphis*, est décrit pour la première fois.

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Spielbereitschaft beim Wisent

Von Erna Mohr

Eingang des Ms. 1. 4. 1967

„Welch fürchterlich Geschöpf, welch schreckende Figur,
welche Muskeln — welche Sehnen! Welche fremde Kreatur!
Wie ein Erden-Behemoth, scheinen seine Knochen Ertz,
seine Hörner würcklich Eisen . . .
Wie so wild und fürchterlich ist sein starker Kopf behaart,
Es vermehrt sein wildes Aussehen sonderlich sein dicker Bart,
Ein gesetzter Mut belebt ihn, ihm ist keine Furcht bekannt,
Er kennt seiner Hörner Kraft, wo er stehet, hält er Stand."

So charakterisiert der alte Ridinger den Wisent in der Unterschrift zu seinem wohlgelungenen Stich eines Wisentbullen. Und Brehm berichtet: „Sein Kopf ist mäßig groß und durchaus nicht plump gebaut . . . der Gang ein rascher Schritt, der Lauf ein schwerer, aber schnell fördernder Galopp. Ältere Tiere erscheinen uns als ernste, fast mürrische, leicht reizbare und jähzornige, jeder Tändelei abholde Wesen".