Studies on Schmauch Bodies. I. The Incidence in Normal Cats (Felis Domestica) and the Morphologic Relationship to Heinz Bodies

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HEINZ BODIES have been known for 75 years (since 1890), but it is only during the past 25 years, particularly after Moeschlin's1 work, that these inclusions have attracted interest.

A number of workers have investigated the clinical occurrence and experimental production of Heinz bodies both in vitro and in vivo; some authors have also outlined species differences in Heinz body formation,2 but relatively few observers3-14 have reported or mentioned the physiologic occurrence of similar, if not identical, erythrocytic inclusions in normal cats. These inclusions, known in veterinary literature as Schmauch bodies, were named after the German pathologist Schmaucha who, as early as 1899, was the first to give a detailed description of their morphology and incidence in normal cats.

Since species differences in the experimental production of Heinz bodies are undoubtedly the most conspicuous and the most interesting aspect of the phenomenon, one would expect that it is also the phenomenon of Schmauch bodies that has been given special attention, at least during the past 20 years. One would also expect that the spontaneous occurrence of Schmauch bodies in normal cats and the striking similarity between Schmauch and Heinz bodies would have been considered of importance for the studies of the spontaneous occurrence of Heinz bodies in humans as found in “idiopathic Heinz body anemia,”15 in “hemolytic anemia of premature babies with the spontaneous development of Heinz bodies,”16 and most recently in “congenital”17 and “hereditary Heinz body anemia.”18 Strangely enough, however, up to the present time, no work has been published in which a detailed comparison between Heinz bodies in humans and Schmauch bodies in cats has been made. This is interesting in view of the fact that cats have been extensively used for the experimental production of Heinz bodies.

The purpose of this paper—the first of a series to be published on Schmauch bodies—is to present the results of studies on the incidence of Schmauch bodies in normal domestic cats, and on the comparative morphology of Schmauch and Heinz bodies. This work is based on a survey of literature, on the count of the Schmauch-body containing red blood cells in 94 healthy cats, and on the analysis of the staining characteristics of these inclusions.

Review of the Literature

In general there have been two groups of investigators concerned with the problem of inclusion bodies in cats: those interested primarily in the

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Table 1.—The Incidence of Schmauch Bodies in Cats According to Data in the Literature

<table>
<thead>
<tr>
<th>Author and Reference</th>
<th>Year</th>
<th>Number of Animals Examined</th>
<th>Percentage of Affected Erythrocytes</th>
<th>Method of Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schmauch³</td>
<td>1899</td>
<td>15</td>
<td>1–80</td>
<td>Methyl violet</td>
</tr>
<tr>
<td>Natscheff⁴</td>
<td>1931</td>
<td>13</td>
<td>0.2–85</td>
<td>Brilliant cresyl blue</td>
</tr>
<tr>
<td>Freifeld et al.⁵</td>
<td>1937</td>
<td>Not indicated</td>
<td>Higher in adult animals</td>
<td>Nile blue sulphate</td>
</tr>
<tr>
<td>Hoffbauer⁶</td>
<td>1937</td>
<td>22</td>
<td>0.1–71.4</td>
<td>Brilliant cresyl blue</td>
</tr>
<tr>
<td>Wirth and Sbratt⁷</td>
<td>1937</td>
<td>2</td>
<td>27.9 and 78.6</td>
<td>Brilliant cresyl blue</td>
</tr>
<tr>
<td>Ottawa⁸</td>
<td>1938</td>
<td>10</td>
<td>0.4–3.8</td>
<td>Brilliant cresyl blue</td>
</tr>
<tr>
<td>Gross et al.⁹</td>
<td>1942/43</td>
<td>Not indicated</td>
<td>10–30</td>
<td>Nile blue sulphate</td>
</tr>
<tr>
<td>Jung¹⁰</td>
<td>1946/47</td>
<td>Not indicated</td>
<td>Scarce (in one cat 80)</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Bock¹¹</td>
<td>1947</td>
<td>Not indicated</td>
<td>Approximately 20</td>
<td>Nile blue sulphate</td>
</tr>
<tr>
<td>Fertman and Doan¹²</td>
<td>1948</td>
<td>Not indicated</td>
<td>Higher in old animals</td>
<td>Brilliant cresyl blue</td>
</tr>
<tr>
<td>Wirth¹³</td>
<td>1950</td>
<td>Not indicated</td>
<td>0.1–71.4</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Schalm¹⁴</td>
<td>1961</td>
<td>Not indicated</td>
<td>0.2–1.0</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Schalm¹⁹,²⁰</td>
<td>1962/63</td>
<td>“Limited”</td>
<td>5–10</td>
<td>New methylene blue</td>
</tr>
</tbody>
</table>

In order to obtain a more clear blood picture of normal animals from the anatomic point of view, and those interested primarily in the experimental production of inclusion bodies from the toxicologic point of view. A survey of the most important investigations of both groups of workers is presented in table 1. It is easily seen that since the initial work of Schmauch³ no more than 12 papers⁴⁻¹⁶ have been published touching in some way or other upon the incidence of inclusion bodies in normal cats. Even in the veterinary literature this subject seems to have been neglected: more than 30 years had to elapse until Natscheff,⁴ in a review on “the incidence and the significance of the vitally stainable erythrocytes of the domestic animals,” confirmed the earlier observations that a certain proportion of erythrocytes of normal cats contained Schmauch bodies. In the recent American veterinary literature the term “Schmauch bodies” has not been used. Schalm in his textbook on Veterinary Hematology, (1961) states that the occurrence of “Howell-Jolly-like bodies” (Schmauch bodies?) in up to 1 per cent of the erythrocytes is common to cat’s blood.¹⁴ However, 1 year later (1962) the same author found that inclusion bodies, distinct from Howell-Jolly bodies but not stained by methyl violet, occurred in 5–10 per cent of clinically normal cats and in half of those with different diseases.¹⁹ In a recent publication, Schalm and Smith²⁰ named these inclusions “erythrocyte refractile bodies” (“ER bodies”) but gave no reference to the contributions of early workers⁵⁻¹³ on the subject.

With the growing development of experimental toxicology the cat has been...
used more and more as an experimental animal especially since the cat is generally recognized as the animal most susceptible to the production of Heinz bodies. Freifeld, Schilowka and Ludwinsky\textsuperscript{5} used cats in their toxicologic investigations and stated that they never found Schmauch bodies in the newborn cats, but did find a small number in young, and a considerable number in adult animals. However, in the European veterinary literature of that time\textsuperscript{6-8} there are more precise data on the incidence of Schmauch bodies, though the number of the examined cats still remains small. Other subsequent reports\textsuperscript{11,12} in which relatively high counts have been observed are listed in table 1.

It is interesting to note that among the authors who used cats as most satisfactory laboratory animals in toxicology research, there are some who seem to have been unaware of the physiologic presence of Schmauch bodies in cats.\textsuperscript{21-23} There are also some works\textsuperscript{24-26} on the blood picture of normal cats in which no mention of the presence of Schmauch bodies has been made at all.

**Animals and Methods**

Ninety-four randomly chosen typical house cats of either sex in apparent good health, from the vicinity of Zagreb, were used in this study. Their age, more or less accurately known only in 63 animals, ranged from 2 days to 12 years; their weight ranged from 0.10 to 4.5 Kg. In 31 animals not even an approximate age was known.

In order to secure conditions as basal as possible, the animals were not narcotized. The blood was collected from the vena saphena magna and the very small drop touched to a glass slide for phase contrast microscopy; 0.5 ml. of the remaining blood was placed into a test tube containing heparin and the dye for supravital staining.

**Phase Contrast Microscopy**

To the small drop of blood placed on a glass slide a coverslip was applied, and observations were made with an oil immersion lens. Some observations were recorded photographically employing repeated exposures at 30-minute intervals.

**Supravital Staining**

Methyl violet or Nile blue sulphate, 0.5 per cent each in normal saline, were used for supravital staining. Equal amounts of blood and dye were mixed in a test tube and allowed to stay for half an hour; then a small drop of the mixture of the dye and blood was used to make a smear. The incidence of Schmauch bodies in the red blood cells was found by counting 1000 cells stained with Nile blue sulphate, since this stain soon proved to give most satisfactory results.

**Results**

The inclusions in the erythrocytes of normal cats may be identified either by phase contrast microscopy (fig. 1) or by supravital staining (fig. 2).

**Morphology in Phase Contrast Microscopy**

Schmauch bodies can easily be demonstrated in the wet, unstained preparation of feline blood using phase contrast microscopy. An affected red cell usually contains a single, mostly rounded, inclusion, but double or irregularly shaped forms may also be seen. These inclusions at times appeared to pro-
Fig. 1.—Phase contrast micrographs illustrating the typical appearance of Schmauch bodies (A) and the changes leading to the spontaneous liberation of the bodies (B) and (C).
Fig. 2.—Nile blue sulphate supravital staining of the erythrocytes of a normal cat. Schmauch bodies are seen in the majority of red blood cells.

Fig. 3.—Heinz bodies from a patient poisoned by m-dinitrobenzene. Compare and contrast with the bodies appearing normally in the blood of cats. (Same magnification.)

trude causing a bulging of the cell, but they were never observed to leave the cells, although many extraerythrocytic bodies are usually found.

The changes leading to the liberation of Schmauch bodies could be followed by observing them under the phase contrast microscope (fig. 1) for a longer time. The inclusion at first seen as an opaque, dark body near the middle of the cell (fig. 1A) or eccentrically, is “moved” to the very periphery of the cell increasing in “translucency” (fig. 1B). At the same time, the affected erythrocyte begins to fade until it becomes a ghost cell consisting of a partly visible cell membrane and within its circumference there is an inclusion body (fig. 1C). The latter in the meantime becomes entirely trans-
Fig. 4.—(A) Photomicrograph of supravitaly stained blood film of a normal cat demonstrating Schmauch-bodies containing erythrocytes and a reticulocyte. (B) Same area as above after decoloration and restaining with May-Grunwald Giemsa, now showing the disappearance of Schmauch bodies but not of reticulocyte.

lucent, looking almost as the miniature of the affected ghost cell itself, but regular in shape and very sharp in outline. Finally the ghost disintegrates completely and the Schmauch bodies are left free in the suspension.

Morphology in Supravital Staining

Both methyl violet and Nile blue sulphate give satisfactory results when used for supravital staining of Schmauch bodies. However, Schmauch bodies are particularly conspicuous by their intense basophilia when stained supravitally with Nile blue sulphate (fig. 2, 4A). They are usually single, generally round, relatively large and almost uniform in size occupying about a third to a fifth of the cell diameter. There is otherwise no change in the staining quality of the cell content.

Comparison of phase contrast preparation with supravitaly stained smears made with the same specimen of the feline blood shows that the inclusions
in unstained preparations are not so strictly confined to the periphery of the erythrocyte as they are in the stained films. When unstained, they also appear to be smaller in size than those demonstrated in the smears stained with supravital staining technics.

The Incidence

Table 2 shows the incidence of Schmauch bodies in the erythrocytes of normal cats. Schmauch bodies were consistently present but varied considerably in number ranging from 0.3 per cent to 96.1 per cent; only in 1 of 94 cats was it not possible to demonstrate Schmauch bodies.

In 63 animals attempts were made to correlate the incidence with age. After the whole sample (N = 63) was divided into three categories according to age (I = up to 1 year, II = from 2 to 5 years, III = more than 5 years), Kruskal-Wallis’s “One-Way Analysis of Variance by Ranks”-test was carried out in order to prove if there were differences between samples. The value obtained was H = 3.597, which, with 2 d.f., is lower than the critical value of the \( \chi^2 \)-test at the significant level \( P = 0.05 \). On the basis of these results it cannot be concluded that in cats of different age there are differences in the number of Schmauch bodies.

Relationship to Heinz Bodies

The inclusions discovered and described in cats by Schmauch show many features identical to those known as Heinz bodies formed in other animals and in humans as a result of the toxic action of a wide variety of chemical compounds. Their common characteristics are as follows: (1) both may be seen as refractile particles in unstained wet preparation; (2) both have a marked affinity to supravital stains (methyl violet, brilliant cresyl blue and Nile blue sulphate); (3) both fail to stain with Romanowsky dyes. The latter property is very important because it makes both Heinz and Schmauch bodies distinct not only from Howell-Jolly bodies and other nuclear remnants, but also from reticulocytes: when a Romanowsky dye is used to restain supravital preparations only reticulocytes but not Schmauch bodies are restained (figs. 4A, B).
DISCUSSION

The results obtained in this work confirm earlier observations that Schmauch bodies may be present in the erythrocytes of normal cats. \(^3\) Moreover, data presented in this paper give sufficient evidence that "endoglobular bodies" as originally named and described by Schmauch \(^3\) are almost invariably present in this species. They were demonstrated in 93 out of 94 randomly examined domestic cats. There is very little information about the factors which might influence the incidence of Schmauch bodies. In 1948, Fertman and Doan \(^1\) stated that they could "confirm the report of German investigators that old animals show these inclusion bodies in a small proportion of circulating erythrocytes in the absence of any known external toxic agent." In the present investigation, however, it was not possible to establish any correlation between the percentage of affected red cells and the age of animals.

Phase contrast microscopy was used in this study in order to exclude any possibility of the influence of supravital examination upon the production of Schmauch bodies. Webster, Liljegren and Zimmer \(^2\) have emphasized the difficulty of finding a solution capable of maintaining red blood corpuscles of various species morphologically unchanged during a supravital examination. It has also been known since the work of Gutstein and Walbach \(^2\) that even Nile blue sulphate itself may produce Heinz bodies. Phase contrast microscopy, however, has also the advantage of direct and adequate visualization of viable erythrocytes floating in their natural environment.

The Schmauch bodies studied in this work showed all the morphologic and staining characteristics described by Heinz in his original communication \(^2\) and by subsequent workers. \(^1,2,5,10,12,27,29\) It seems evident, therefore, that the inclusion bodies found physiologically in cats are identical with the Heinz bodies formed by some toxic compounds in humans and in animals. Moreover, the liberation of Heinz bodies after disintegration of the ghost cell membrane, as described by Bratley, Burroughs, Hamilton and Kern \(^10\) in pyrodin poisoning in dogs, is strikingly similar to the liberation of Schmauch bodies as observed in this study.

In view of the fact that the Heinz body formation is due to a partial destruction of some portion of the red cell, it would be expected that an appreciable amount of hemoglobin is also lost to the cell by the formation of Schmauch bodies. However, even when Schmauch bodies are present in the majority of the erythrocytes of normal cats, and also in spite of the fact that they are usually much larger in size than the Heinz bodies in humans (compare fig. 2 and 3), no hemolytic anemia in cats with the high Schmauch bodies count has been evidenced. This is in sharp contrast to what has been observed in humans in whom the presence of Heinz bodies in such a great number would almost always be regarded as a very serious state. It is this pathophysiologic distinction that necessitates further studies and warrants, at least for the time being, yet another eponym in biologic literature.

SUMMARY

Inclusions in the erythrocytes of normal cats, called Schmauch bodies, have been demonstrated by means of phase contrast microscopy and supravital
staining. A very wide range of their incidence, but no correlation with the
age of animals has been noted. By their identical staining reactions and
common morphologic characteristics Schmauch and Heinz bodies are quite
similar, although they might be produced by quite different mechanisms.

SUMMARIO IN INTERLINGUA

Per medio de microscopia a contrasto de phase, e tincturation supravital,
inclusiones esseva demonstrate in le erythrocytos de cattos normal. Iste
inclusiones esseva designate como corpores de Schmauch. Esseva notate un
extensissime variabilitate de lor incidentia, sed nulle correlation con le etate
del animales studiate. Corpores de Schmauch e corpores de Heinz ha identic
reactiones tincturatori e commun characteristicas morphologic, sed il pare
probabile que le duo es producite per molto differente mechanismos.

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