CERTAIN CHARACTERISTICS OF THE LEUKOCYTES OF GUINEA PIG BLOOD WITH PARTICULAR REFERENCE TO THE KURLOFF BODY

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ANYONE about to begin a study of the reaction of the blood of guinea pigs to an experimental procedure is confronted with the lengthy task of determining, first of all, the normal blood picture for this animal. Leukocytes of the guinea pig are unlike leukocytes of the human being in many details. Reports are in disagreement and have not been adequately summarized. Especially controversial is the question of the causation and significance of the azurophilic cytoplasmic inclusion which was first described by Kurloff and which bears his name. The Kurloff body seems important because it occurs in the blood cells which may be most involved in recovery from tuberculosis; namely, the mononuclear leukocytes. Blood cells were examined from more than 500 animals, including normal guinea pigs and guinea pigs which were being used in chemotherapeutic experiments on tuberculosis. These observations are presented with a critical review of the literature on the subject. Thus an effort has been made to establish a normal blood picture for the guinea pig, with special attention to the Kurloff body.

LITERATURE

The differential distribution of leukocytes as reported by twenty-one investigators has been summarized in table 1. The approximate ratio of the percentage of granulocytes to the percentage of mononuclear leukocytes accepted as normal for man (60:40) is roughly reversed in the guinea pig. There is considerable variance in the individual interpretations of the staining reaction of the granules in the polymorphonuclear leukocytes. Myelocytes were reported as occurring regularly in the blood stream by Klieneberger[1] and Buchheim.[2] Lucia and Lucia[3] stated that eosinophilic granules of the guinea pig are oval or round; while Ringoen[4] expressed the belief that they are round at first, but later assume oval patterns. Extreme variability in the percentages of eosinophilic granulocytes was reported.[5, 6] Mesnil[7] and Weinberg and Séguin[8] stated that eosinophils are phagocytic.

There is general agreement that the basophilic granulocytes of guinea pigs do not resemble those of human blood. Basophilic granules are large, oval, and coarser than those found in either rabbit or human blood. Furthermore they are said to swell, rather than to dissolve in water, and to give a positive oxidase and a negative peroxidase reaction.[9] Maximow and Bloom[10] expressed the belief that the histogenous and the hematogenous mast cells are independent types and are even more differentiated from one another in the guinea pig than in man. The granules of the tissue mast cell were derived from the nucleus, while those of the hematogenous mast cell were thought to differentiate from a basophilic protoplasm, independent of any special nuclear participation.[11, 12]

Several hematologists have expressed the belief that the monocytes of guinea pig

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TABLE 1.—*Differential Distribution of Leukocytes in Blood of Normal Guinea Pigs*

Investigator	Polymorphonuclear leukocytes, per cent*	Lymphocytes, per cent	Large mononuclears, per cent	Eosinophils, per cent	Basophils, per cent	Kurloff cells, per cent	Notes
Bender and DeWitt	35–60	35–55	5–8	3–4	1		Transitionals 0–3%
Burnett	31.5	47.3	10	10.7	0.37	0–2	
Burnett	47.0	50.0		2.0	1.0	0–2	
Frey	26–31	60–65	4–5†	1–2	1–3		
Goodall	37.0	60.0		3.0			
Gulland	52.0	42.0		6.0			
Howard	43–79	16–36	0.8–6.6	2.0–33.6	0–1.2		
Hunter	16–36			0.2–33.6			
Jolly and Aouna	10–55	53.0	53% of 9.5–10μ†	1.0			
Kanthook and Hardy	62.0 (8μ)	24 (6μ)	11†	2.3 (10μ)	0.7 (8μ)		
Klieneberger	9–47	33–88	0.4–4.0†	10.0	0–1.0		Unclassified to 3%.
Klieneberger	38.5	46.8		13.0	0.85		33% small lymphocytes, 18.5% large lymphocytes
Krocke and Garver	41.8	45.3	8.4†	4.8	0.7		
Kurloff	40–50	30–35	15.0–20.0	1.0 (quoted as 10%)	0.5	15.0–20.0	
Ledingham						4.0–11.0	
Loewit	52.5	38.5	3.0†	3.3			
Lucia and Lucia	15.42	74.39	4.9	2.4		2.06	Used oxidase reaction to differentiate
Lyons and van de Carr	37.9	49.0	7.0†	3.11	0.8	5.0	Unclassified 0.2%
Meyer	36.26	57.08	4.25†	1.69	0.72		
Mezinecoue	22–30	45.0	3.0†	7.0	2.0		
Schilling	25–30	65–70	1–2†	1.0–3.0	1–3	1–2	
Scholz	47.0	38.0			5.0		
Stäubli	(Writes that mononuclears exceed polymorphonuclear leukocytes. No figures)			0.5–35.0	2 or less		

* Called variously acidophils, indulinophils, heterophils, pseudo-eosinophils, or polymorphonuclear leukocytes.

† Listed as monocytes, not large mononuclears.

blood have a coarser nuclear reticulum than those of human blood.[13] Many have stated that the proportion of large lymphocytes to small lymphocytes is much greater in the guinea pig than in man and that the nuclei of the larger types are

frequently U- or S-shaped. Burnett[14] spoke of early lymphocytes wherein the nuclei are almost divided into two smaller nuclei. The rather controversial question as to whether Kurloff bodies occur in monocytes as well as in lymphocytes or in both of these mononuclear cells rests largely on the classification of the large mononuclear cells. Kurloff bodies occur in 5 to 20 per cent of the mononuclear cells of the guinea pig.

The Kurloff body. Kurloff and Foà and Carbone described these bodies,[15–17] independently, in 1889. Kurloff expressed the belief that they are secretory products elaborated by the cells, which first appear as isolated punctate granules, enlarge gradually to the size of the nucleus, and then break through the protoplasm and are discharged. He estimated that cells containing these bodies constitute 15 to 20 per cent of the leukocytes. The number was not increased by splenectomy. He said that the granules are common to many species but that the aggregation of them into the form of these bodies is peculiar to the guinea pig and families related to it. Foà compared these bodies to parasites but failed in his attempts to cultivate them and reported negative reactions for fat, albumin, starch, glycogen, and iron. He observed that they were more numerous in the spleens of pregnant than of other guinea pigs. Burnett regarded them as degenerated nuclei of mononuclear leukocytes, occasionally found in polymorphonuclear leukocytes as phagocytosed particles.

Cesaris-Demel[18, 19] said that Kurloff bodies first appear in the 3 day old guinea pig, and he believed them to be secretory products. He was not familiar with the work of either Kurloff or Foà; they are therefore sometimes called Kurloff-Foà-Demel bodies.

Ledingham[20] studied the blood of four related species of the Caviidae but found no Kurloff bodies. He believed, in 1906, that they were blood parasites or inclusion bodies of infectious origin, but he later changed this opinion.

Maximow was convinced that Kurloff bodies do not occur in all guinea pigs. Accordingly, he became a follower of Ledingham's hypothesis of parasitism. Ciaccio[21] presented evidence that Kurloff bodies are degenerated nuclei. A nucleus, he said, divides and one part becomes smaller, stringy, and pyknotic and then disappears. Howard[22] regarded the Kurloff body as an artefact.

Patella,[23] using arsenic and quinine, failed to rid the guinea pig blood of Kurloff bodies but said that pregnant guinea pigs were markedly "infected." Schilling[24, 25] considered Kurloff bodies as phagocytosed blood elements undergoing degeneration. They were thought to be especially prevalent in sites of destruction of blood and increased in infections. Pappenheim and Ferrata,[26] Pappenheim[27] and Weidenreich[28] believed them to be coalesced azure granules. Ross[29] described a life cycle of Kurloff bodies. Purple granule inclusions increased in size and became dumbbell-shaped, then rod-shaped. Flagella appeared at both ends of the rod and the whole then split longitudinally and finally was discharged from the cell. Kolmer[30] was the first to assert that Kurloff bodies disappear in a castrated animal. Grunner[31] found increased numbers of Kurloff bodies with age. He reported an occasional one in a neutrophilic premyelocyte.

The first reports of the in vitro culture of Kurloff body cells were made by Awro-

row and Timofejewsky.[32] They reported that after ten hours most of the lymphocytes with Kurloff bodies had developed pyknotic nuclei and then degenerated. Those lymphocytes without Kurloff bodies hypertrophied and became phagocytes. In two days all the granulocytes had degenerated. A few of the Kurloff body cells which remained hypertrophied and became macrophages, whereupon their Kurloff bodies disappeared.

Since Kurloff bodies gave positive reactions to iron stains, Woodcock[33] concluded that they were phagocytized red blood cells. Senez[34] reported an occasional Kurloff body in the cells of the fetal spleen. Bender[35] stated that Kurloff bodies were nucleated when stained with neutral red stains. He could not obtain positive iron reactions but did show positive results with fat stains.

Vasaturo[36] counted more Kurloff bodies in animals that had lead poisoning and in those in which experimental anemia had been induced than in normal animals. Wada[37] found no Kurloff bodies in castrated animals. He regarded them as a colloid or an albuminoid substance which was dependent on the hormonal state of the animal.

Lucia and Lucia[3] reasoned that Kurloff bodies stained with a vital stain because they were phagocytosed degenerating cells and that the spongy crenated appearance of these bodies was the result of digestion which was followed by a condensation to form the typical Kurloff body. They observed a negative correlation between the number of Kurloff bodies and the number of lymphocytes and so concluded that these bodies occurred only in monocytes.

Alexieff and Joukoff[38] related the development of Kurloff bodies to disturbances of lipoid metabolism. They reported that these bodies disappeared from the blood of 60 castrated guinea pigs but reappeared when "spermine" was injected. After six months, however, Kurloff bodies did not develop in such castrated animals on the injection of spermine. Semenskja[39] stated that Kurloff bodies were increased in famine, avitaminosis, pregnancy, puberty, and hemorrhage. He regarded them as lymphocytic secretions derived from azure granules and suggested that they play some role in immunity. Etzel[40] claimed to have found Kurloff bodies in Brazilian Caviidae and called them "Kurloff bodies of rodents." Liggeri[41] reported positive reactions to fat stains and increases of numbers in vitamin deficiencies and in fasting conditions. Chilla[42] studied Kurloff bodies in animals having chronic and acute alcoholism. Hinteregger[43] found that the numbers of Kurloff bodies increase with increase of the basal metabolism of animals. He called them azure granules. Iibuchi[44] found that Kurloff bodies increase in typhus fever.

Lazzeroni[45] claimed that Kurloff bodies were increased in numbers whenever destruction of red cells occurred; he believed therefore that they were phagocytosed red blood cells.

Spink[46] investigated the reported relationship between the eosinophilic leukocyte and the Kurloff bodies but concluded that the number of Kurloff bodies was directly proportional to the number of monocytes in the blood and not to the number of eosinophils.

Natucci[47] recorded fewer Kurloff bodies after hysterectomy, and Frey[48] stated that they were toxic products. Studying the blood of guinea pigs poisoned with

azoimide, Frey obtained an increased number of Kurloff bodies with the induced leukopenia. Since the cells comprising the myelogenous system were decreased, he drew the conclusion that the Kurloff cells were derived from either the lymphatic or the reticulo-endothelial system. A few of the Kurloff cells in his study gave a positive peroxidase reaction. These, he believed, were monocytes which had phagocytosed a polymorphonuclear leukocyte, giving rise to the Kurloff body. Mochkovski[49] said decisively that Kurloff bodies were parasites and accordingly designated them "Ehrlichia (Rickettsia) Kurlovi." Babudieri[50] found no increase of the number of Kurloff bodies in the blood of guinea pigs that had experimental anemias and concluded that they were degenerating lymphocytes. Hutyra, Marek, and Manninger[51] first suggested the possibility of homology with Russell body cells or Auer bodies.

Tosatti[52] attempted to culture Kurloff bodies. He claimed that they disappeared in castrated animals but reappeared on administration of sex hormone, thereby supporting the earlier observations of Kolmer,[30] Alexieff and Joukoff,[38] Wada,[37] and Cilotti.[53]

The last extensive report on Kurloff bodies was a review published in 1940 by Ledingham,[54] who concluded that they were related to the sex hormones, contrary to his expressed opinion thirty-four years previously that they were blood parasites. The 1940 report also stated that the Kurloff cell was not a monocyte but a lymphocyte, because it did not phagocytose colloidal dyes. He denied any relationship of Kurloff bodies to azure granules. He stated, too, that with administration of sex hormone the number of Kurloff cells was increased but there was no increase in the number of azure granules. He said that the newborn guinea pig has azure granules but no Kurloff bodies.

Interpretation of the literature. From this review it is obvious that the many authors disagree on the appearance, the occurrence, and the significance of the Kurloff bodies. The only special staining reaction agreed on was the vital red stain. There was agreement too in the observation that these bodies exhibit brownian movement, as do virus inclusions.

There was difference of opinion with regard to species specificity, cellular specificity, whether the Kurloff bodies were always intracellular, and whether they occurred at all in fetal blood or in blood of newborn guinea pigs. The failure to find Kurloff bodies in counting 100 leukocytes in a smear hardly justifies the conclusion that they were absent from the blood of such guinea pigs. The belief that these bodies are artefacts may well be discarded, for the several varieties may occur in the same field and in unstained and unfixed preparations. The small pale accessory forms which occur in young guinea pigs before the large solid or stringy bodies appear could not be accounted for if Kurloff bodies were degenerating nuclei.

Increased numbers of Kurloff bodies were reported in many deficiency and diseased conditions which seem superficially unrelated.

The only correlation which was found between these diverse reports is that in all of the conditions cited as causing an increase of Kurloff bodies, there were also fewer circulating lymphocytes. Kurloff bodies were increased in conditions resulting in granulocytosis where accompanied by relative lymphocytopenia (in experi-

mental anemias and toxic states and with the eosinophilia of trypanosomiasis) and in tuberculosis where large leukocytoid lymphocytes are found but with active lymphopenia. Increases were reported in a few conditions where the relative proportion of granulocytes was decreased but because of leukopenia the total number of lymphocytes was also decreased (as in the pregnant guinea pig). Even the increased numbers of Kurloff bodies reported to occur with age and their increases on administration of hormone were all proportional to the total decrease of lymphocytes. Lucia and Lucia observed a negative correlation between the total count of lymphocytes and the number of Kurloff bodies and considered this as evidence that the Kurloff bodies are phagocytosed particles occurring only in monocytes.

Thus in any condition in which there was a proportionate increase of the number of mononuclear cells containing Kurloff bodies, there was also a decrease of the total number of mononuclear leukocytes. This suggests that the amount of Kurloff material in the body may tend to remain at a relatively constant level.

To test the theoretical deduction that the amount of Kurloff material may remain constant, and because of the uncertainty as to the nature of the Kurloff body, a further study of this interesting cell inclusion was undertaken. An exceptionally large number of animals were available for study, most of which were being used to test various chemicals for their possible deterrent effect on experimental tuberculosis. Since the mononuclear leukocyte may be concerned in recovery from tuberculosis, and since the Kurloff body is peculiar to mononuclear leukocytes of the guinea pig, it was hoped that observations would disclose some correlation between the incidence or structure of the Kurloff body and this disease process.

METHODS AND MATERIAL STUDIED

Blood from guinea pigs was obtained by pricking an ear vein or directly from the heart. Thin smears were made on specially cleaned new slides and were stained as follows:

American Wright's or Grübler's	1½ minutes
Buffer (added until a metallic scum formed)	5 minutes
Giemsa (1 drop to 5 cc. of distilled water freshly diluted)	7 minutes

Imprints of the spleen, lymph nodes, and bone marrow of the femur were stained with the same staining combination but for slightly shorter time intervals. A few duplicate blood smears known to contain a high percentage of Kurloff bodies were stained for fat, iron, and amyloid, and with Ehrlich's triacid stain. In addition, blood samples were stained supravitally with Janus green and vital red.

One hundred to 500 leukocytes were counted on each blood smear and at least 500 cells were counted in each bone marrow preparation. In many instances the number of lymphocytes, of mononuclear leukocytes, and of Kurloff cells per 100 leukocytes and also the number of Kurloff cells per 100 mononuclear leukocytes were charted. The percentage of mononuclear leukocytes which contained azure granules and the relative number of mature Kurloff bodies seen in mononuclear leukocytes were determined.

Samples of the blood of many normal adult males and females and of fetuses and newborn were examined for Kurloff bodies. In addition to these, blood smears and

imprints of bone marrow were made from more than 500 male guinea pigs which were used in chemotherapy experiments. Some of these animals had hemolytic anemia, others cyanosis, some showed extreme shifts to the right or to the left in the maturity of heterophils. Animals with variable degrees of toxicity, many stages of guinea pig tuberculosis, and a number with hyperplastic thyroids induced by the chemotherapeutic agents were studied.

The estrual cycle of the guinea pig is completed in fifteen to sixteen days. Blood smears of 3 virgin females were made at regular short intervals during two estrual cycles. In addition, blood smears were made of 3 pregnant guinea pigs at regular intervals to term. One virgin female received 4 rat units* of estrin and another 40 rat units, intraperitoneally, five days a week for four weeks. Blood smears were made at the time of each injection. Three adult male guinea pigs were castrated.

TABLE 2.—*Percentage Distribution of Polymorphonuclear Leukocytes of Guinea Pigs and Man by Number of Lobes in Nuclei*

Lobes	Guinea pig	Man*
1	2	5
2	7	35
3	19	41
4	27	17
5	30	2
6	10	
7	4	
8	1	

* After Arneth.[55]

Blood smears were made at intervals up to 188 days after orchectomy and the changes in the Kurloff bodies were observed.

Thus an attempt was made, first, to determine the normal leukocyte picture and, second, to investigate the relationship which may exist between the hormone state, metabolism, intercurrent disease, and the Kurloff bodies. Finally, cultures of the spleen and the buffy coat of centrifuged heart blood of animals known to have a high Kurloff count were attempted in living chick embryos.

GENERAL OBSERVATIONS

Polymorphonuclear leukocytes of the guinea pig show a more extensively lobulated nucleus than do those of man. An average of 5 typical hemograms, counting as individual lobes those separated by a filament, is given in table 2. This tendency to increased lobulation is evident to a lesser extent in the eosinophils and basophils of the guinea pig. While the majority of basophils of the guinea pig have the large, dark purple, round or oval granules described in the literature, occasional angular ones with an orchid-staining reaction are seen. Angular granules gave the impression that if they were pushed together their edges would fit like pieces of a puzzle.

* One rat unit equals 10 international units.

Many of the large mononuclear cells of the guinea pig exhibit the azure dust, the high cytoplasmic-nuclear proportion, vacuoles and other cytoplasmic characters that classify them as monocytes according to criteria for human monocytes; yet their nuclei are typically lymphocytic, containing coarse, trachychromatic, irregular chromatin strands and exhibiting poor differentiation of chromatin from parachromatin. Likewise the scanty blue cytoplasm characteristic of the large human lymphocyte may, in the guinea pig, surround a nucleus which is stretched to an S or Z shape and which appears evenly reticular like the nucleus of a monocyte. The divergence of opinion as to how some of these large cells should be classified is readily understandable.

However, the most striking difference between mononuclear leukocytes of guinea pigs and those of man is the existence of Kurloff bodies in the cells of the guinea

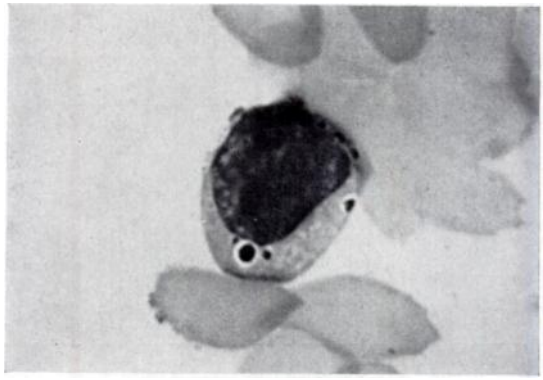

FIG. 1. SEVERAL SMALL EARLY KURLOFF BODIES (×1500)

pig. Records were kept of every variation encountered and of their frequencies in all the normal and experimental guinea pigs studied.

OBSERVATIONS OF THE KURLOFF BODIES

Kurloff bodies were very rare in the blood of the guinea pig fetus and newborn. At approximately 2 months of age, however, the variety and number of Kurloff bodies assumed adult proportions, concomitant with the development of the adult differential distribution of the leukocytes. By following changes in the Kurloff bodies, it was possible to classify the various types as progressive stages in their development (figs. 1 to 8).

Kurloff bodies obtained from cardiac blood of the fetus were apparently all alike. They were about a third the size of the nucleus of a lymphocyte; they were round and of a pale solid red color, in the cytoplasm of typical lymphocytes, but not always enclosed in a vacuole.

Lymphocytes of the newborn guinea pig showed, in addition, granules which were usually pale red. Many of them were enclosed in small individual vacuoles (fig. 1). Sizes of these granules ranged from less than 1 micron to that of the

medium-sized Kurloff body observed in the fetus. These bodies often appeared as a red ring, as though their size had increased faster than their substance. They probably are the "isolated punctate granules" or "specks" mentioned by Kurloff,[15]

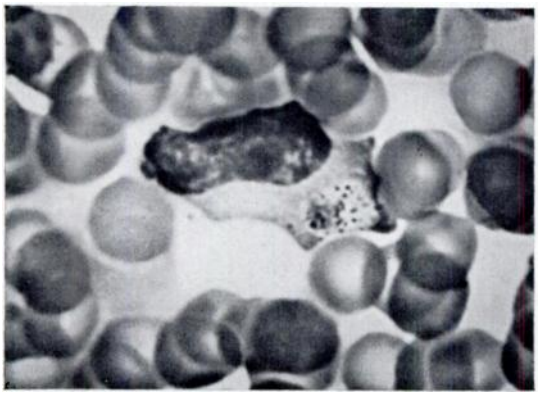

FIG. 2. GRANULES COALESCING TO FORM A MATURE KURLOFF BODY (×1500)

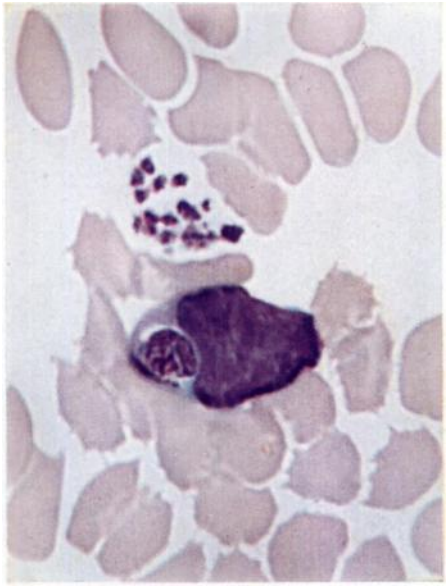

FIG. 3. MATURE KURLOFF BODY; THE COMMON TYPE (×1500)

the "small inclusions" of Senez[34] and Wada,[37] the "accessory Kurloff bodies" of Bender,[35] the "subsidiary bodies" which Ross[29] said resembled "polar bodies." Pappenheim and Ferrata[26] agreed that the small and large forms are functional phases of the same cell. Azure granules in vacuoles do occur in mononuclears of other animals than the guinea pig.

By the time the adult blood picture is attained, several of these homogeneous red Kurloff bodies have become very large, resembling the typical cell inclusions of certain virus diseases. This is the most common type of a Kurloff body (figs. 3, 4, and 5). In the development of the most common type of Kurloff body in the adult guinea pig it is also not unusual to find a large vacuole containing 10 or more of the individual red granules, separated from each other by fibrous strands which may be the remnants of the walls of the vacuole (fig. 2). Sometimes these fibers

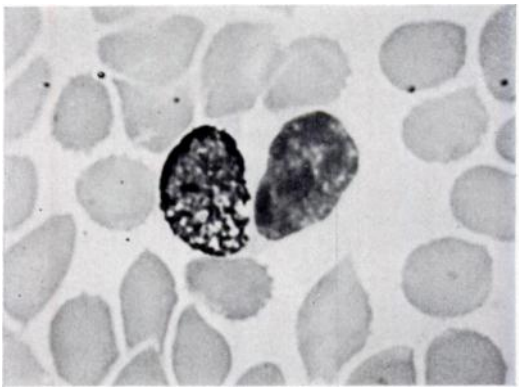

FIG. 4. MATURE RED KURLOFF BODY BECOMING PURPLE AT THE PERIPHERY (×1500)

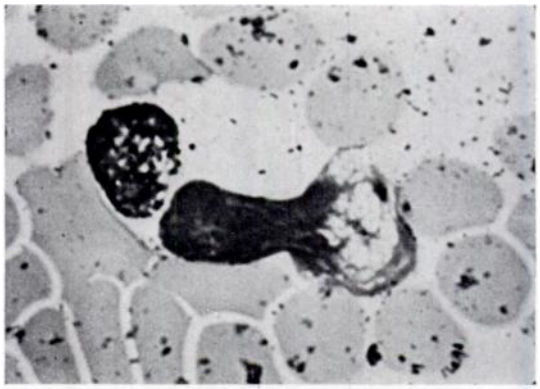

FIG. 5. MATURE KURLOFF BODY WITHIN A "BASKET" CELL (×1500)

appeared to be connected to the granules; they probably constitute the flagella hitherto described.[3, 29] Rodlike appearances and spiremes[3, 56] may be explained as stages in the final fusion of these granules to form the solid large Kurloff bodies.

A later stage in the development of the Kurloff body is derived from this solid red stage. The staining reaction of the body changes from light red to dark red, then to purple around the periphery (fig. 4). By the time the whole Kurloff body is purple, it is no longer a solid, colloid-looking body but has broken up into numerous, nearly uniform, crystalline-appearing diploid or tetrad small round bodies

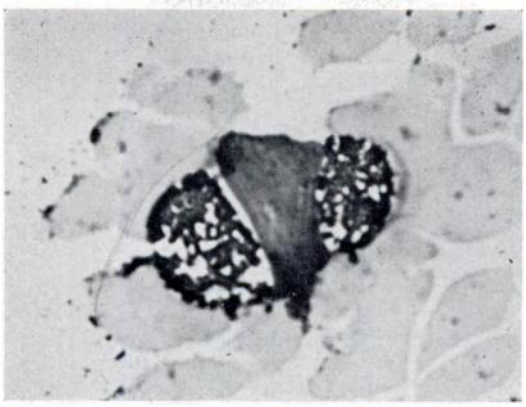

FIG. 6. TWO KURLOFF BODIES IN THE LATE PURPLE STAGE OF DEVELOPMENT WITHIN THE SAME CELL (×1500)

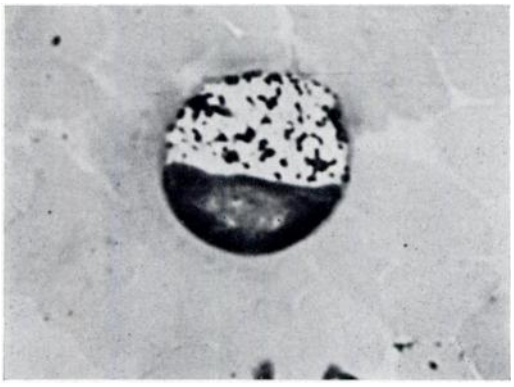

FIG. 7

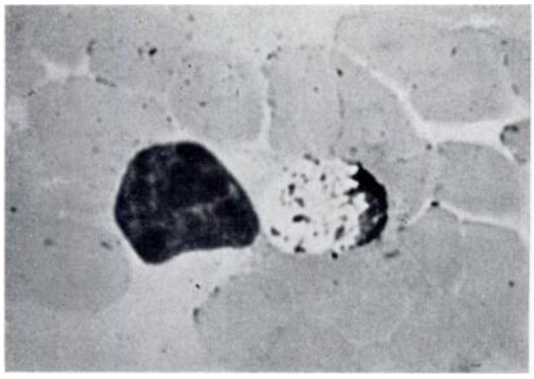

FIG. 8

FIGS. 7 AND 8. KURLOFF BODIES IN LATE STAGES WITH KURLOFF SUBSTANCE DISAPPEARING (×1500)

(fig. 6). Indeed, these resemble nothing so much as the morula state of a blood sporozoon. Some spleens of normal guinea pigs contained large numbers of these final stages. These late purple forms cannot be found in the castrated animal after four to six weeks. In evenly stained films of the blood of normal guinea pigs, purple and red stages often appeared in the same field. Burnett has stated that the larger a Kurloff body is, the more deeply it is stained. Rather, it would seem that the larger and older a Kurloff vacuole is, the more deeply do its contents stain. A large, solid, young Kurloff body not yet in a vacuole takes a light red stain. As the body shrinks and darkens, the vacuole continues to enlarge. All gradations (figs. 6, 7, and 8) were seen, in the adult, ranging from the large vacuoles packed with purple, crystalline-looking bodies (fig. 6) to those which, through loss or disintegration of these individual bodies, appear to contain only large empty vacuoles. A vacuole was often seen containing just enough purple, dustlike material to identify it as a Kurloff body.

Kurloff bodies occurred free in the plasma in some dried smears. They were either the solid or stringy red forms, often with a purple edge, or the small round or oval purple bodies. In every instance in which free Kurloff bodies were found, a damaged mononuclear leukocyte, either a "basket" cell or a lymphocyte with an opened vacuole, was identified near by. In one slide of a normal guinea pig's blood there were 4 such Kurloff bodies lying in or, more likely, on polymorphonuclear leukocutes. Figure 5 shows a degenerating basket cell with a Kurloff body in the cytoplasm.

The great majority of Kurloff bodies occur in lymphocytes, but small, medium, or large Kurloff bodies occasionally occur in monocytes. These cells in guinea pigs have a comparatively coarse chromatic reticulum and often have U-, J-, or S-shaped nuclei. There is another type of monuclear cell in guinea pig blood which occasionally contains Kurloff bodies. This is a large cell, with a round or indented nucleus having a reticular arrangement of chromatin and a distinct nuclear membrane. These have characteristics of reticulo-endothelial cells. An unusual feature of them is an especially large amount of finely dispersed acidophilic material in their cytoplasm. They probably are the cells mentioned in the literature as circulating myelocytes which contain Kurloff bodies.[2, 15] Downey,[57] however, regarded them as monocytes and the acidophilic material as azure "dust." But there are many typical monocytes without this predominance of acidophilic material in their cytoplasm. In these cells, this red material seems to accumulate and to form first a red body which later becomes the typical vacuolated Kurloff body. There is the possibility that these cells may be just Ferrata cells, now known to be the result of injury. However, it would appear more likely that they are transition forms between reticulo-endothelial cells and mature lymphocytes or monocytes.

Kurloff bodies are always present in a variety of stages in normal, adult guinea pig blood. A few occur in bone marrow, and most are found in imprints of the spleen. Although they abound in the mature lymphocytes of the circulating blood, they are exceedingly rare in lymph nodes. Phagocytosed red blood cells were clearly differentiated from Kurloff bodies in the cells of spleen imprints of animals that

had hemolytic anemias. There were no increases in counts of Kurloff bodies in blood smears of such anemic animals.

Kurloff bodies were sometimes found in lymphocytes which contained 2 nuclei, and sometimes 2 large Kurloff bodies were observed in a cell in which the nucleus was compressed between them (fig. 6). Rodlike or spireme-like stages in the development of the Kurloff body suggested a morphologic similarity to the prophase or metaphase stage of mitosis. Occasionally the Kurloff body was attached by connecting strands to the nucleus (though this has been denied by Kurloff) and suggested a relationship to spindle fibers. Spindle fibers would stain red with the acid fuchsin in Ehrlich's triacid stain, but Kurloff bodies were not stained red. Heterophil granules were stained, but not azure granules, by this staining combination. Thus it would seem that Kurloff bodies are not related to cell divisions and that they could be azure granules capable of hypertrophy and dissolution.

In other species of animals, plasma cells occur in the splenic sinuses which may contain Russell bodies resembling the large red inclusion type of Kurloff body.[58] Plasma cells appeared in the blood of some of the guinea pigs receiving chemotherapy, but they were never seen to contain Kurloff bodies (or Russell bodies).

Auer bodies, such as those which occur in lymphoblasts and myeloblasts of the spleens of human beings having acute leukemia, resemble only the rather rare rodlike stage occasionally seen in the development of the Kurloff body. However, vaccinia inclusions resemble the more common solid red stage of Kurloff bodies, stain with neutral red, and exhibit brownian movement just as the Kurloff bodies do.

The Kurloff bodies were no more prevalent in the blood of guinea pigs which were resistant, naturally, to the tubercle bacillus, than in the blood of more highly susceptible guinea pigs. This was, considering the paucity of disease at necropsy, which occurs consistently in about 10 per cent of our stock strain of guinea pigs after experimental infection, regarded as evidence of the animal's resistance. Likewise no increase occurred in guinea pigs rendered immune to diphtheria by an excessively large amount of diphtheria toxoid. The number of Kurloff bodies per 100 leukocytes in the blood of animals dying of tuberculosis was not increased. But since such animals have more granulocytes and fewer mononuclear leukocytes than normal animals, the number of Kurloff bodies per 100 mononuclear leukocytes was greater than in healthy animals.

Several of the chemotherapeutic agents tested induced decreases of the proportion of granulocytes to 5 per cent or less. In these animals there were consistently fewer Kurloff bodies per 100 mononuclear leukocytes, and they were usually the kind found in the newborn. In certain of the guinea pigs in which a shift to the left of the neutrophil picture developed or a shift to the right (nuclei often with 15 or 16 lobes) had occurred, the number of Kurloff bodies had not changed. This was also true in smears in which excessive pyknosis, karyorrhexis, or leukocytoid lymphocytes could be demonstrated.

In castrated animals, as in the chemically induced lymphocytoses, the structure of the Kurloff bodies approached that in the newborn guinea pig. After four to six

weeks, large, dark red or purple forms could rarely be found; by ten weeks after castration there were still some pale medium-sized red Kurloff bodies and more of the granule-like bodies, with rounder, younger looking lymphocytes as the host cells. Twenty-seven weeks after castration as many as 16 per cent of the monucl ar leukocytes contained these early Kurloff bodies. No mature forms could be found; only the small, pale, round or ringed forms previously observed to be the precursors of the mature Kurloff body. Castration, then, does not cause the disappearance of Kurloff bodies but seems to prevent their normal maturation.

Smears of a number of pregnant guinea pigs were made at frequent intervals through term. In certain instances a high percentage of Kurloff bodies was recorded; yet there were no indications that pregnancy, per se, induced any real changes in the number of cells containing mature Kurloff bodies. This is at variance with data hitherto reported. A few observations were made on the influence exerted by the hormone estrin on the number of Kurloff bodies. Four rat units and 40 rat units, respectively, were administered daily, five days a week for four weeks, to adult female guinea pigs. The data were of interest in that again, as in pregnancy, the percentage of mature Kurloff bodies per 100 leukocytes remained relatively constant throughout the time the hormone was given.

COMMENT ON KURLOFF BODIES

Experimental evidence indicates that Kurloff bodies are not phagocytosed elements, sex secretion, immune bodies, Russell bodies, degenerated nuclei, or artefacts. On slides known to contain large numbers of Kurloff bodies, these bodies remained unstained by fat, iron, or amyloid differential technics. Kurloff bodies do not seem to be concerned in the multiple hemopoietic potencies of the lymphocyte. It is usually not the cell containing a Kurloff body that becomes a macrophage in tissue cultures. This is not the variety of lymphocyte that becomes the epithelioid cell of a tubercle.

Kurloff bodies occur in a higher proportion of mononuclear cells where there are fewer mononuclear cells per 100 leukocytes. Likewise the lowest percentages of Kurloff bodies per 100 mononuclear leukocytes occur where the relative proportion of mononuclears is high. This suggests that Kurloff bodies may represent a secretory or excretory product of the mononuclear cell which tends to remain constant in quantity.

A promising field for further investigation would seem to be to utilize this unusually large azure-staining inclusion to elucidate the physiologic characteristics of mononuclear cells. The visible ripening of the Kurloff granules is definitely retarded by castration and by most disease processes. It is an attribute of maturity and good health in the guinea pig. Toxic drugs do not increase the number of granules and retard rather than augment their development. The possibility exists that they may be symbiotic parasites, but, in our institution at least, all normal guinea pigs have Kurloff bodies. To date, attempted cultures in chick embryos have yielded negative results. It is only the morphologic resemblance to parasitic or virus stages that suggests an infectious origin. Convincing evidence that the presence of Kurloff bodies in any way modifies the response of the animal to any experimental procedure was not recorded.

SUMMARY

A critical review of the literature on the leukocytes of the guinea pig has been presented, with added observations from studies of the blood cells of more than 500 guinea pigs.

Special studies of the Kurloff inclusion body were made on these animals to obtain information regarding its origin and significance because this entity occurs in the mononuclear leukocyte, which is a cell of great interest in the pathogenesis of tuberculosis.

The normal blood picture of the guinea pig was discussed and summarized in terms of the blood picture of the normal human being.

CONCLUSIONS

It may be concluded that the leukocytes of the guinea pig differ from the leukocytes of the human being in the following particulars: (1) a lower percentage of polymorphonuclear leukocytes; (2) an increased lobulation of the polymorphonuclear leukocytes; (3) larger, variably shaped, water-insoluble granules of the basophilic leukocytes; (4) a higher proportion of mononuclear leukocytes; (5) mononuclear leukocytes which are less readily classifiable specifically as monocytes or lymphocytes, and (6) the presence of Kurloff inclusion bodies in 2 to 20 per cent of the mononuclears of all normal mature guinea pigs. There is presumptive evidence to support the belief that the Kurloff bodies are secretory or excretory products of the cells in which they occur. The evidence in support of this presumption is based on the following observations: (1) Kurloff material tends to maintain a constant quantitative level in the blood stream of the mature animal no matter what the count of the mononuclear leukocytes may be, (2) Kurloff bodies develop in an orderly pattern which is related to the physiologic development of the animal and is changed when certain glands are removed, and (3) the inclusion is not a constant associate of any particular pathologic entity.

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