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Title	CLINICAL AND EXPERIMENTAL STUDIES ON THE ERYTHROCYTES WHICH INCLUDE JOLLY'S BODIES IN HORSES
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Citation	Japanese Journal of Veterinary Research, 8(1-4), 1-11
Issue Date	1960
DOI	https://doi.org/10.14943/jjvr.8.1-4.1
Doc URL	https://hdl.handle.net/2115/4666
Type	departmental bulletin paper
File Information	KJ00002373258.pdf



CLINICAL AND EXPERIMENTAL STUDIES ON THE ERYTHROCYTES WHICH INCLUDE JOLLY'S BODIES IN HORSES

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(Received for publication, December 10, 1959)

INTRODUCTION

The small point-like violet-reddish bodies observed in the erythrocytes by the use of Giemsa staining have been called HOWELL-JOLLY'S bodies or JOLLY'S bodies for short. It has been thought that these small bodies are some of the remainder of the nuclei.⁶⁾ Up to this time, these erythrocytes with the small bodies (abbreviated "J cells") have been said to belong to the category of so-called young erythrocytes such as erythroblasts, polychromatic erythrocytes, erythrocytes with basophilic granules and reticulocytes.

Observing the reports on the erythrocytes with these small bodies in horses, one learns firstly, that JOEST and JÄHNICHEN found the appearance of small anaplasma-like bodies at the rate of 2 per cent of the erythrocytes in blood of osteomalacia horses, although from blood of normal horses also they found a small number of small bodies in the erythrocytes identical to the former. They decided that the small bodies may be the same as the so-called JOLLY'S bodies. After that, in the blood of horses affected with infectious anemia, BAARS, SCHAAF and also KRAL observed a few such small bodies, but on the other hand, KASAI et al. did not find them at all. According to KOHANAWA'S report on the examination of the blood of 12 normal horses, no J cells were detected, but WIRTH found 442 and 114 J cells per cubic millimeter of blood in 2 normal horses, respectively. ISHII stated that J cells appeared at the rate of 6.6 per cent in normal colts and at the rate of 13.3 per cent in 2~4-year-old horses. The number of the appearances was about 100 per cubic millimeter of blood. On the other hand, the cells were observed at the rate of 20.0~66.6 per cent in infectious anemia horses, the number being about 300 per cubic millimeter in the average at various stages of the course of the disease. In the above noted reports, J cells were investigated only in respect to the rate of appearance and number of appearance as minor parts in the blood findings of normal or certain diseased horses; true form and

the clinical significance were not discussed at all.

In view of the present condition, the author undertook some clinical and experimental investigations on these cells.

MATERIALS AND METHODS

Horses for experiments: For the clinical observations use was made of 170 clinically healthy adult horses, 12 clinically healthy colts bred in the vicinity of Sapporo and 42 horses affected with infectious anemia which attended our veterinary clinic.

The horse for experimental observation was a clinically healthy four-month-old colt.

Methods of observations: Smears of blood or bone marrow fluid from the above described horses were made as thin as possible in usual manner and were dried quickly. After fixation by methanol, they were stained with Giemsa solution and also sometimes were examined by supravital staining with brilliant cresyl blue. In the counting of J cells, an ocular-blind was used and the rate of appearance of J cells was recorded as number of appearances per one hundred thousand erythrocytes.

RESULTS OF THE EXPERIMENTS

I. Conditions of Appearance

1. Appearance of J cells in the clinically healthy horses

The rate of appearance of J cells in the healthy adult horses is shown in table 1. It will be understood from this table, that the minimum and maximum numbers of appearances were 1 and 45, respectively. The average number was 10.1. From these findings, it was clarified that in the blood of normal horses, J cells are always present. In the correlation

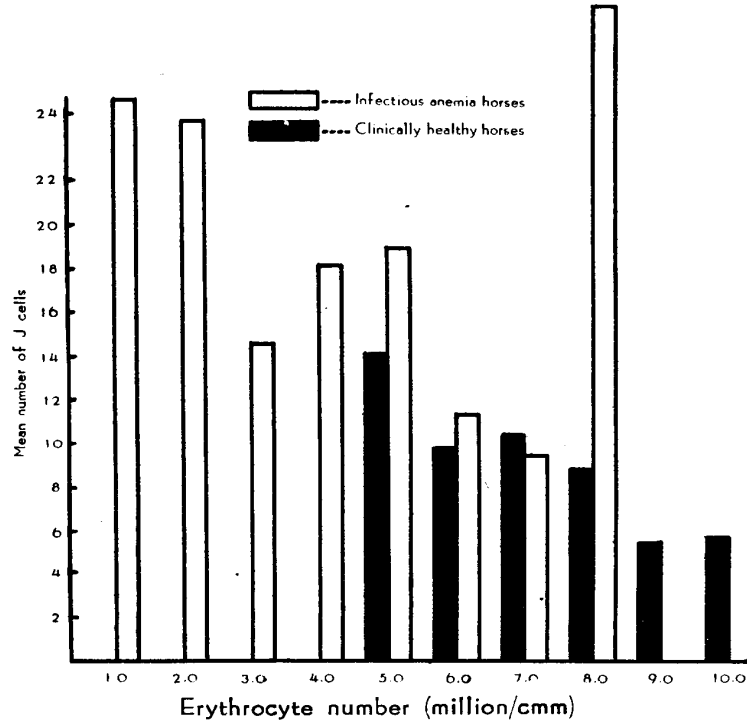
TABLE 1. *Rate of Appearance of J Cells in Clinically Healthy Horses*

	NUMBER OF J CELLS APPEARED*								
	1~5	6~10	11~15	16~20	21~25	26~30	31~35	36~40	41~45
No. of cases	61	50	28	11	5	8	2	2	3
Rate of appearance (%)	35.9	29.4	16.5	6.5	2.9	4.7	1.2	1.2	1.8

* Number per 0.1 million of erythrocytes

between the number of appearances of J cells and the number of cases, there were 61 cases (35.9 per cent) that showed number of appearances of 1~5 cells. They were most prevalent. The second prevalence was in the group of 6~10 cells (29.4 per cent) and third was in the group of 11~15 cells (16.5 per cent). That is to say, in accordance with the increase of the number of appearances, the rates of appearances decreased antagonistically; there were only 7 cases over 31 cells. In chart 1, the correlations between the number of appearances of J cells and the number of erythrocytes in 170 cases were graphed. The numbers of appearances of J cells in the 2 groups of over 9 millions in erythrocyte

CHART 1. *Relation between Numbers of J Cells Found and Erythrocyte Numbers*



numbers were 5.5 and 5.8, respectively; these 2 values were markedly smaller than those of the other 4 groups. In the other 4 groups of 5~8 millions in erythrocyte numbers, they were counted from 8.9 to 14.1; it was the highest in 5 millions level and the lowest in 8 millions level in erythrocyte numbers. In general, the tendency of increase was observed in the groups of smaller number of erythrocytes. In the 12 clinically healthy colts which are 7~210 days old, the cases over 10 which is the mean value of healthy adult horses, were 3 (25.0 per cent) and the cases under that value were 9 (75.0 per cent). The range and the mean value of the number of appearances were 1~42 and 11.5, respectively.

It may seem that there are no characteristic differences of appearance in comparison with the appearances in adult horses.

2. Appearance of J cells in horses affected with infectious anemia

The rate of appearance of J cells was examined in total 160 samples obtained from 42 cases affected with infectious anemia, having 1~8 millions in erythrocyte number. The result is shown in table 2. The numbers of appearance in 153 samples excepting 7 samples, in which J cells could not be found at all, ranged 1~112 cells with mean value 18.7. Especially, the cases which showed from 1 to 30 appearances numbered 125 cases; these cases occupied the greater part of those which showed appearance of J cells.

Furthermore, it may be thought that if many more erythrocytes were counted, in

TABLE 2. *Rate of Appearance of J Cells in Horses Affected with Infectious Anemia*

	NUMBER OF J CELLS APPEARED*								
	0	1~10	11~20	21~30	31~40	41~50	51~60	61~70	71~112
No. of cases	7	72	35	18	6	6	3	6	7
Rate of appearance (%)	4.3	45.0	21.9	11.3	3.8	3.8	1.9	3.8	4.3

* Number per 0.1 million of erythrocytes

the 7 cases where no J cells were observed, J cells might be able to be found in small number. In the blood of infectious anemia horses, the mean value of the number of appearances increased by 8 over that of clinically healthy horses, the range of their distribution being more wide than that of the clinically healthy horses.

The relation between the number of appearances of J cells and erythrocyte number in horses affected with infectious anemia is indicated in chart 1. From the data in this chart, in general, a slightly increasing tendency for J cells to appear in the group of small number of erythrocytes was observed, but it can not be said that there are characteristic differences in appearance between the group with a small and that with a large number of erythrocytes.

For the purpose of discussion of influences of fever attack upon the appearance of J cells, the relation between body temperature and the number of J cells observed is recorded as in table 3.

TABLE 3. *Relation between Number of J Cells Appeared and Temperature*

TEMPERATURE (°C)	NUMBER OF J CELLS APPEARED*					
	0~10	11~20	21~30	31~40	41~50	51~112
Over 40.0	8	6	4	1	1	2
40.0~39.0	28	13	6	2	2	5
Under 39.0	43	16	8	3	3	9

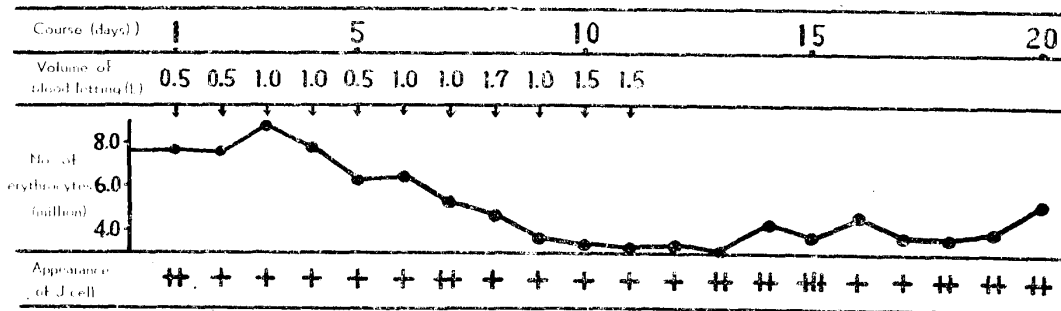
* Number per 0.1 million of erythrocytes

From the data, no apparently constant tendency could be noticed at all. Also, the changing phases of J cells in 5 infectious anemia horses repeating typical fever attack were examined in course, but they underwent different changes. So, a plain relation between them was not observable.

3. Appearance of J cells in a colt rendered anemic by blood-letting

A clinically healthy four-month-old colt, was subjected to blood-letting at the rate of 500 ~1,700 ml per day, and the condition of the appearance of J cells was observed during about 30 days before and after operation. The data of the experiment are shown in chart 2 where it will be seen that slightly increasing tendency was observed during the period from the 13th day, the animal becoming most anemic at the time near it's recovery from anemia. The maximum number of appearance was under 30; that is, the distribution changed in the range of the appearance of J cells observed in the clinically healthy colts described already. On the 14th and 17th days of the experiment, reticulocytes which appear usually very rarely in the blood of the horses, were observed in small number.

CHART 2. Finding in a Colt Rendered Anemic by Blood-letting



Note: + ~ ## show 1~10, 11~20 and 21~30 J cells per 0.1 million of erythrocytes, respectively.

From this finding, it is thought that this period corresponds to the most active time in the reproduction of the erythrocytes, but on the basis of the above data, it can not be said for certain that, although J cells increased slightly, the tendency in the appearance of J cells was characteristic.

4. Comparison of the appearance of J cells between circulating blood and bone marrow fluid

Comparison of the appearance of J cells accompanying circulating erythrocytes and the erythrocytes of bone marrow fluid was conducted in clinically healthy horses and also in horses affected with infectious anemia. The data are shown in table 4.

In 7 cases of 10 belonging to the clinically healthy horse group, the number of appearances of J cells in the erythrocytes of the bone marrow fluid increased more markedly than that of circulating blood. The differences between them were 2~30; it was 8.3 on the average. In 3 other cases, the number of appearances increased in the circulating blood over that of the bone marrow fluid; the range of the difference was from 1 to 8, being 4 on the average. In the case of horses affected with infectious anemia, the blood of the bone marrow fluid contained more J cells than circulating blood, in 9 out of 10 cases. The variation in the findings was 11~121 and the average number was 49.2; this is larger in comparison with that of clinically healthy horses. In the peripheral

TABLE 4. *Number* of J Cells Appeared in Peripheral Blood and Bone Marrow Fluid*

CASE NO.	CLINICALLY HEALTHY		CASE NO.	INFECTIOUS ANEMIA	
	peripheral	bone marrow		peripheral	bone marrow
1**	22	19	11**	18	17
2	12	14	12	14	52
3	5	11	13	69	84
4**	41	33	14	5	82
5**	9	8	15	7	43
6	0	2	16	15	136
7	11	12	17	11	48
8	20	26	18	3	14
9	13	46	19	6	47
10	2	10	20	25	92

* Number per 0.1 million of erythrocytes

** Superior in peripheral blood to in bone marrow fluid

TABLE 5. *Relation between Numbers of J Cells and Erythroblasts*

SAMPLE NO.	NO. OF ERYTHROCYTES (million/cmm)	NUMBER OF APPEARANCES*	
		J cells	Erythroblasts
1	4.16	2	1
2	4.50	8	2
3	3.25	13	2
4	3.40	14	2
5	3.62	15	2
6	3.45	18	1
7	3.74	25	1
8	5.70	30	1
9	3.28	31	16
10	1.04	39	12
11	1.25	55	10
12	2.47	68	4
13	1.83	72	10

* Number per 0.1 million of erythrocytes

blood, only in 1 case out of the 10 did there appear a slightly higher value than that of bone marrow fluid in the number of J cells. In view of the above findings, in the clinically healthy horses, it may be said that there are scarcely any differences

in the number of appearances of J cells in circulating blood and in bone marrow fluid. Also in respect to the horses affected with infectious anemia, it can not be asserted that the number of J cells which appear in the bone marrow fluid is always larger than that in the peripheral blood.

5. Correlation between the appearance of erythroblasts and the number of J cells

In the blood of the 170 clinically healthy horses, no erythroblasts were found in any case. On the other hand, on the examination of 160 samples from 42 horses affected with infectious anemia, erythroblasts were detected in 13 samples among them. In table 5, the correlation between the number of erythroblasts which appeared and the number of J cells is tabulated.

J cells appeared always in much greater number than erythroblasts but they did not always increase and decrease in parallel with the rise and fall of the number of erythroblasts.

6. Rate of appearance of erythroblasts with JOLLY's bodies

By the use of smears of bone marrow fluid obtained from 30 horses affected with infectious anemia, 200 erythroblasts were examined for the estimation of the rate of appearance of the erythroblasts containing JOLLY's bodies. The results are shown in table 6.

TABLE 6. *Number of Appearances of Erythroblasts with J Bodies*

	NUMBER OF APPEARANCES OF ERYTHROBLASTS WITH J BODIES(%)						
	0	1~2	3~4	5~6	7~8	9~10	Over 10
No. of cases	9	12	3	2	2	1	1
Rate of appearance (%)	30.0	40.0	10.0	6.7	6.7	3.3	3.3

Although the number of appearance of J cells in the peripheral blood of horses affected with infectious anemia was 18.7 on the average to one hundred thousand erythrocytes, the mean number of appearance of erythroblasts with JOLLY's bodies was 2.5 per cent; the latter value was much greater than the former.

From these findings, it is evident that, there are enough erythrocytes with JOLLY's bodies in bone marrow fluid to supply the J cells found in peripheral blood in number.

II. Morphological Observations on J Cells

1. Size of J cells

The diameter of 305 J cells obtained from clinically healthy horses was measured. The data are shown in table 7.

The mean value of the diameter was 5.2 μ . This is a slightly smaller value than that

for diameters of erythrocytes of clinically healthy horses, viz. 5.52μ , as has already been reported by the present author⁹⁾. The mean diameter of J cells obtained from 36 horses affected with infectious anemia was 5.87μ . This differs little from the mean value of the diameter of erythrocytes, viz. 5.84μ , of the horses affected with infectious anemia.

From the above results, it may be said that J cells whether obtained from clinically healthy or from infectious anemia horses were not very different in size from those of erythrocytes of clinically healthy horses or infectious anemia horses.

TABLE 7. *Size of J Cell*

	DIAMETER OF J CELL (μ)						
	3.75~ 4.50	4.50~ 5.25	5.25~ 6.00	6.00~ 6.75	6.75~ 7.50	7.50~ 8.25	Over 8.25
Clinically healthy	27	144	109	24	1	.	.
Affected with infectious anemia	22	217	203	142	39	13	1

2. Size of JOLLY's bodies

The measurements were conducted of the size of 500 JOLLY's bodies in the blood of horses affected with infectious anemia.

There were found 116 point-like bodies (33.2 per cent) under 0.75μ , 320 bodies (64.0 per cent) between $0.75 \sim 1.5 \mu$ and 14 bodies (2.8 per cent) between $1.5 \sim 2.25 \mu$ in size. No JOLLY's bodies over 2.25μ in size were observed.

In size only, these JOLLY's bodies were differentiated distinctly from the nuclei of erythroblasts.

3. Number of JOLLY's body in a J cell

There was only one JOLLY's body in the 1,711 J cells obtained from the blood of the clinically healthy horses; there were no J cells containing over such body. On the other hand, in the horses affected with infectious anemia, out of 2,996 J cells from 160 samples of blood smears obtained from 42 affected cases, there were found 15 J cells which included two JOLLY's bodies and one cell with three JOLLY's bodies. In smears of bone marrow fluid, J cells which contained two JOLLY's bodies were observed very rarely amongst those from the clinically healthy horses, but in those from the infectious anemia, J cells which contained four JOLLY's bodies were found.

4. Finding of J cells by supravital staining

By supravital staining with brilliant cresyl blue, the JOLLY's bodies were dyed blue or slightly purplish blue. It was found that the bodies fixed in the stationary position, do not move in the cells; that is, when the cells were moved or the forms of the cells were

changed by pressure to cover glass, the J cells always moved with the JOLLY's bodies fixed at the proper position.

In the peripheral blood of clinically healthy horses and horses affected with infectious anemia, the erythrocytes containing both JOLLY's bodies and so-called supravital stainable substances such as *substantia reticulo-filamentosa* or *substantia methachromatico-granulosa* were not found at all in any of the smears of all cases observed. But in the smears from the bone marrow fluid, although the number of appearances was very small, J cells with supravital stainable substances were found.

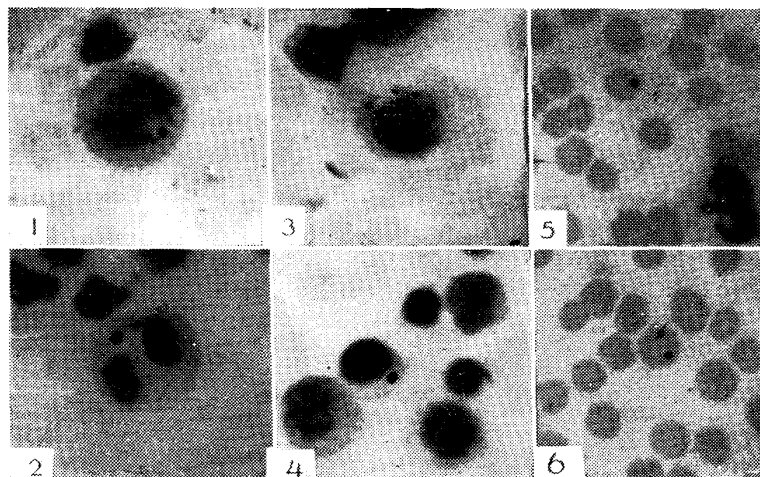
CONSIDERATION

To summarize, from the above results, the present author can not agree with the opinion that the erythrocytes with JOLLY's bodies are cells belonging to the category of so-called young erythrocytes such as has been thought up to this time.

From the fact that the erythroblasts in the bone marrow fluid contained so many JOLLY's bodies corresponding to the number included in the erythrocytes of the peripheral blood, it is thought that the birth of these small bodies occurs by protrusion and isolation of the nuclear substances in the course of the nuclear division, not in the course of elimination of the nucleus from cells.

According to the experiments of WIRTH and his co-workers¹¹⁻¹⁵⁾ on blood-letting in domestic animals, in the blood of horses, a few J cells were always present throughout the whole course of the experiments but they were not increased especially by blood-letting; in dogs, although various kinds of young erythrocytes

*Various Figures of Erythrocytes and Erythroblasts
with JOLLY's Bodies*



Figs. 1 & 2. JOLLY's bodies at the time of nuclear division
Figs. 3 & 4. Erythroblasts with JOLLY's bodies
Figs. 5 & 6. Erythrocytes with JOLLY's bodies

appeared in great number, the appearance of J cells increased only slightly; in pigs, no increase in number of J cells was found at all in contrast with the marked increase of various kinds of young erythrocytes, and in goats and sheep, slight increase of J cells appeared. Furthermore, in cattle, J cells did not increase but in cats, they appeared in comparatively large number followed numbers of polychromatic erythrocytes and erythroblasts. WIRTH and his co-workers thought that the J cells were one sort of new-born young erythrocytes, however in their data, it was clear that the conditions of the appearance of J cells not only in horses but also in other domestic animals did not parallel with the conditions of the appearance of young erythrocytes. Already by many workers, it has been proved that in the splenectomized animals, J cells appear soon after splenectomy and then do not appear during several years.

In this instance, this fact can not be reconciled with the opinion as that J cells belong to young erythrocytes. Not only from the findings in routine morphological examinations, which are very important for judgment whether a cell is young or old, but also from the data on the conditions of the appearance, the present author would like to express the opinion that J cells are one kind of poikilocytes, including JOLLY's bodies, which originated from some part of the nuclear substances in the course of nuclear division. Further they are adult cells which passed through the common course of maturation as well as the normal erythrocytes in the peripheral blood.

SUMMARY

The erythrocytes including JOLLY's bodies in horses were investigated clinically and experimentally. The conclusions arrived at may be summarized as follows.

1. Erythrocytes which include JOLLY's bodies were always present in the blood of horses. The mean number of appearance per one hundred thousand erythrocytes was 10.1 in the clinically healthy horses and 18.7 in the horses affected with infectious anemia, respectively.

2. In continual observations of a colt being rendered anemic by blood-letting, a slight tendency of increase in the number of appearances of erythrocytes with JOLLY's bodies was observed during the period from the maximum anemia to near the time of recovery from anemia.

3. Difference in the number of appearances of erythrocytes with JOLLY's bodies between peripheral blood and bone marrow fluid was not remarkable in the clinically healthy horses; however in the infectious anemia horses, a slight increase in number of JOLLY's bodies found was noted in the bone marrow fluid.

4. There was no special relation between the number of appearances of erythroblasts and the number of appearances of erythrocytes which include

JOLLY's bodies.

5. There were no differences in size between the erythrocytes with JOLLY's bodies and the erythrocytes with no JOLLY's body from the normal horses or from the horses affected with infectious anemia.

6. Erythrocytes with both JOLLY's bodies and so-called supravital stainable substances were not found at all in the peripheral blood of clinically healthy horses and horses affected with infectious anemia.

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