Proceedings 4th World Rabbit Congress, 10-14 October 1988, Budapest Hungary, Vol. 2, 551-566

INVESTIGATION TO THE TEMPERATURE BEHAVIOUR OF RABBITS IN PYROGEN TESTS

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The investigation of veterinary and human preparates concerning pyrogen contaminations are an important detail of quality control.
To Siefert and Godau (1985) pyrogens are heatresistant dialysable Oligo -,poly - and Lipopolysaccharides, which mostly derive from pathogen and non - pathogen bacterias and in case of bacterial origin they are named endotoxines. Applied in small quantities (< 1mg/kg body weight) they cause riger and fever. In respectively high dosage the symtoms extend to heavy shock and death.
By reason of bacterial contamination pyrogens are to be found everywhere. They reached a great significance as contaminations in the production of pharmaceutic preparates. They occur as well in the start materials and starting substrates as in the production and reaction sphere.

Problem

The task of the present review is the exact valuation of the temperature behaviour of various rabbit strains in preliminary investigations of the pyrogene test.
In existing Literature reviews are given only incomplete or no assertions concerning used races and strains.
In the national and international Literature are missed also hints to the influence of age and body weight on the normal temperature of the raboit. The body weight is to be reviewed differenciated concerning the moment of setting in of the animals into the experimental station and the test beginning.
The investigations concerning the age of the animals mostly refer to the moment of NaCl - injection.

The injection of a pyrogen standard solution in all trials has been accomplished stereotypically three days later, so that this factor of a structure change can be accepted as constant.

Literature

As preliminary test for the real preparate test for pyrogene contaminations are to be accepted the inquiry of the normal temperature, the temperature deviation after intravenious injection of a pyrogenefree NaCl solution and the inquiry of the temperature increase after injection of a pyrogen standard (DRESSEL,1966). The normal temperatures found by various authors are sum up in table 1 :

Table 1 : Normal temperature limits of rabbits on the basis of various authors reports (to DRESSEL, 1966)

Author		Normal temperature °C
Siebert a. Mendel	(1923)	39,05
Charonnat and Lechat	(1952)	39,0
Kuna et.al.	(1946)	38,0 - 39,0
Molitor et.al.	(1946)	37,8 - 39,0
Rudat and Junghans	(1954)	38,9 - 39,8
Valyi - Nagy and Kelenty	(1959)	38,4 - 39,5
Chiosa et.al.	(1960)	38,7 - 39,8
Tennent et.al.	(1954)	37,8 - 39,0
Desperak et.al.	(1960)	38,5 - 39,7
Dressel	(1966)	38,0 - 39,4

To LANGE (quoted in DRESSEL, 1964) the by thermoelectrical measuring established body temperatures of rabbits are lower, because in this method the animals are permanent fixed. This causes a temperature decrease. Using mercury thermometers the animals can freely move between the measurings. Here is registrated the normal body temperature.

The different conceptions of some authors to the both methods are to be seen. But the temperature differences are low in both methods.
Also all highly allowable limits for the normal temperature of rabbit are differently valuated by various authors. Valyi - Nagy and Kelentey (1959) even start from seasonale depending variations of the normale temperature :

Spring	:	39,1 - 39,5 °C
summer	:	39,0 - 39,3 °C
autumn	:	38,8 - 39,0 °C
winter	:	38,4 - 38,8 °C

Kraus (1981) refers to the influence of life weight and age on the trials and their results. This author describes the great connection of environmental conditions and the life weight development. Under trial conditions shall to be proved a better life weight development than under production conditions.
The animals should also have a certain age (WH and KAL > 80 d), because an earlyer test beginning, for instance in the youth stade, can cause an injury of the trial results.
Because of technological - economical reasons the optimal use moment seems to be in this limit, in which the daily cumulative life mass increase has a maximum.

Material and methods

The test is to be carried out corresponding to the test prescription at healthy animals of 1,5 to 3,5 kg by rectal measuring of the body temperature. The box temperature is approximately 20 °C. The temperature difference between box and test room is to be permilled at most 2 °C. Environmental charges like optical and acustic irritations are to be kept at a distance of the animals before and during the test.
The rabbits are to submited to the preliminary test at the same day time like the tests.

At a minimum of 3 days for a time at least 4 hours in intervals of 30 minutes the body temperature is to measure. Rabbits which by using of thermoelectric measuring instruments on the 3rd day show body temperatures below 38,0°C or above 39,4°C (new 39,6°C) can not be used for the test.

The following animals were available for the present investigations :

1. White New Zealand rabbits
2. Californian rabbits
3. Little Chinchilla rabbits (only 2 trials)
4. Pyteka rabbits (crossing population, new breeding)

The animals derive from two privat breeding farms (Pyteka, Little Chinchilla) and one state farm (White New Zealand, Californians).

Each rabbit gets 10,0 ml pyrogen - free NaCl-injection-solution per kg body weight. Animals, which in the following measurings in the interval of 60, 90, 120, 150 and 180 min. show an increase of body temperature of more than 0,4°C must not be used for the real preparate test. Within the preliminary test for pyrogen sensibility instead of the test solution are injected 10,0 ml of a pyrogen standard solution intravenous. For producing of this pyrogen standard corresponding to the declaration is to be soluted in isotonic pyrogen-free NaCl - injection solution. Rabbits with a temperature increase of below 0,8°C must not be used for the preparate test, too.

In the present investigation the animals were tested only over a NaCl-injectiom solution and a pyrogen standard solution in order to quarantee comparability.

The parameters, which were analysed, will be showing in the discussion part.

Into the investigations were enclosed at all 405 animals of the mentioned lines. Breeding and trial - mediated deficiencys were immediately selected.

Within the correlation calculation was used the correlations coefficient to BRAVAIS with the formula:

$$r = \frac{\sum_{i}^{n} (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2 \sum (y_i - \bar{y})^2}}$$

The calculations of the regression analysis were carried out on the basis of the following formulas corresponding to the both independent parameters x and y :

$$by = r \cdot \frac{Sy}{Sx} = \frac{SP}{SQ_x}$$

$$bx \equiv r \cdot \frac{Sx}{Sy} = \frac{SP}{SQ_y}$$

Results and discussion

For the strain specific investigation has been worked with the genotypes Californian, White New Zealand and Fyteka.
The test results given in table 2 were obtained :
Table 2 : Test results of the strains Californian (KAL) White New Zealand (WN) and Fyteka

Parameter	$\bar{x}$ KAL	$\bar{x}$ WN	$\bar{x}$ Fyteka
body weight at setting in(g)	2302	2617	2211
body weight at trialstart(g)	2492	2657	2314
ear length (cm)	10,7	11,5	10,5
vein visibility coefficient(P)	8,9	10,0	9,5
SP NaCl(normal temperat.) °C	39,5	39,5	39,4
at NaCl(temperat.change,°C)	0,18	0,19	0,17
SP standard solution (°C)	39,4	39,3	39,4
at standard solution(temp.increase) °C	1,14	1,07	0,99
age at NaCl-injection (d)	125	176	177

Because the time between setting up to trial start can be two - three weeks both parameters were inclosed into the trial analysis. By this way an analyse concerning eventually notjustified body weight increase is possible. Besides the length it was given a coefficient for visibi - lity of the ear edge vein within a 10-point-scale.
The start parameter NaCl gives information on the normal temperature before NaCl-injection.
Possible temperature changes (temperature increase) are registrated by the Δt NaCl-parameter. The same information is to be given on the start parameter standard and Δ t standard (temperature increase).
In the table it is to be seen, that White New Zealand because of their relative high body weight have unambiguous advantages in the ear length. These advantages are remunerated by a high average number of points in the parameter visibility of veins. Relative good normal temperatures bring all three strains before NaCl-injection.

An interesting comparison gives the percentual break-down of the obtained normal temperatures limits in investigations of DRESSEL (1966) and KERSTEN and ZAHN (1963) concerning the own results :

Table 5 : Comparison of the obtained basic temperature limits of various rabbit strains (in %)

Strain	n	Temperature limits <39,4 °C	39,5-39,9°C	>40°C
Californian	51	45,1	43,1	11,8
WhiteNew Zealand	41	31,7	65,9	2,4
LittleChinchilla	52	71,2	28,8	0
Pyteka	252	65,9	29,8	4,3
Σ	396	60,3	35,1	4,6
KERSTEN/ZAHN		20,5	45,6	33,9
DRESSEL		98,5	1,3	0,2

The average parameters show, that might be possible a strain specific influence on the temperatures.

Whereas the little Chinchilla and Pyteka are located in the limit below 39,4°C with 71,2 % or 65,9 % of all animals, this part at the Californian and White New Zealand is widly below 50 %. In this lines 34,9% (Californian) and 68,3% (White New Zealand) did not reach the mentioned in AB - GDR (medicine law) normal temperature limit of below 39,4°C. At KIRSTEN and ZAHN (1983) the normal temperature limit mentioned in AB - GDR could only be reached by 20,5 % of the used rabbits.At DRESSEL (1986) this part amounts to 98,5% of all used animals.

The relative great differences of the here shown results let us suppose the fact, the several authors used different lines. Already ZAHN (1983) demands, that a larger number of rabbit strains are to be investigated concerning their suitability for pyrogen tests.

A further interesting comparison results from the question, whether male or female animals of the same strain have different basic temperatures.

The following table shows a comparison of the both sexes within different lines.

Table 4 : Sex comparison of the both sexes within different strains (WN, KAL, Pyteka) - NaCl-Injection-normal temperature °C

Genotype	n	♀ $\bar{x}$	♀ s_{n-1}	n	♂ $\bar{x}$	♂ s_{n-1}
Californian	54	39,00	0,38	15	39,47	0,36
White New Zealand	14	39,44	0,25	26	39,48	0,39
Pyteka (white)	74	39,42	0,35	59	39,19	0,33
Pyteka (marten col.)	44	39,35	0,35	48	39,20	0,37
Pyteka (all)	117	39,38	0,34	106	39,22	0,36

The parameters are showing the clear tendency, that male animals have the lower basic temperature before NaCl-injection. It is generally noticed, that Californian - and White New Zealand rabbits lie above the demanded by AB - GDR maximum temperatures (Californian 39,00°C).

The following normal temperatures could be obtained in comparison of the both sexes before application of pyrogene - standard - preparate.

Table 5 : Sex comparison concerning the basic temperatures with different strains (WN, KAL, Pyteka) normal temperature C - Standard-Injection -

Genotyp	♀ n	♀ $\bar{x}$	♀ $s_{\bar{x}}$	♂ n	♂ $\bar{x}$	♂ $s_{\bar{x}}$
Californian	34	39,56	0,33	15	39,02	0,20
White New Zealand	14	39,37	0,46	25	39,27	0,36
Pyteka (white)	74	39,52	0,33	59	39,35	0,40
Pyteka(marten col.)	44	39,44	0,31	47	39,37	0,45
Pyteka (all)	117	39,47	0,33	105	39,37	0,42

Also in this comparison the male animals tendend clearly for lower basic temperatures. It is to be seen, that nearly all female genotypes lie above the fixed in AL-GDR normal temperature limits.
Neglecting the genotype factor it was possible to subdivide a part of the animals into different age groups.
Table 6 gives an overlook over the measured parameters in the different age groups.

Table 6 : x - parameters of the test animals in different age groups (strains WN, KAL, Pyteka - in days -)

Parameter	<150	151-160	161-170	171-180	181-190	> 190
body weight trial start (g)	2459	2262	2317	2610	2628	2435
body weight setting in (g)	2278	2198	2288	2525	2540	2249
ear length (cm)	10,76	11,15	10,65	11,05	10,74	10,25
vein visibility coeff. (P)	8,94	9,65	9,65	9,81	9,69	9,27
start parameter NaCl (°C)	39,52	39,28	39,38	39,46	39,49	39,51
Δt NaCl (°C)	0,20	0,09	0,09	0,24	0,15	0,25
start parameter stand. (°C)	39,45	39,25	39,31	39,29	39,35	39,49
Δt standard (°C)	1,13	1,06	1,04	1,09	0,97	0,87
age NaCl (d)	125,7	155,4	165,8	177,2	181,6	196,5
FG (n-2)	68	18	25	30	37	24

A tendency is clear to be seen, that animals within the age group of 151 - 160 days with an optimum body weight show a very good ear development and in their normal temperature they lie below the temperature of the other age groups. Also the next group (161-170 days) can show good results in this connection. In the next year this trial is to berepeated within the lines, which was not possible in this material because of the to small quantity of randoms.
Within the line and the age group in the further progress were charged the correlative connections between some meanful parameters (table 7-9).

Table 7: Correlation - and regression coefficients between different parameters within the strain Californian

Connection	F	r	b_x	b_y
bodyweight trial start- body weight setting in	57	$0,630^{+++}$	$0,762^{+++}$	$0,904^{+++}$
body weight trial start- ear length	57	$0,670^{+++}$	$0,002^{+++}$	$220,410^{+++}$
body weight trial start- age NaCl	57	$0,629^{+++}$	$0,035^{+++}$	$11,324^{+++}$
ear length - vein visibility coefficient	57	$0,252^{++}$	$0,305^{++}$	$0,148^{++}$

Table 8 : Correlation - and regression coefficients between different parameters within the strain White New Zealand

Connection	F	r	b_x	b_y
body weight trial start- body weight setting in	38	$0,955^{+++}$	$0,996^{+++}$	$0,911^{+++}$
body weight trial start- ear length	36	-0,012	-0,0002	-0,278
body weight trial start- age NaCl	38	$0,590^{+++}$	$0,012^{+++}$	$29,748^{+++}$
ear length - vein visibility coefficient	36	0,133	0,036	0,469

Table 9 : Correlation - and regression coefficients between different parameters within the strain Lyteka

Connection	F	r	b_x	b_y
body weight trial start - body weight setting in	65	$0,674^{+++}$	$0,534^{+++}$	$0,852^{+++}$
body weight trial start - ear length	65	0,159	0,0003	70,563
body weight trial start - age NaCl	65	$0,321^{+}$	$0,021^{+}$	$5,846^{+}$
ear length - vein visibility coefficient	65	0,009	0,014	0,006

In the present material is to be seen a certain line specific for special connections or parameters. That's why the strain Californian is recomended mostly intense concerning it's quantitive parameters body weight trial start to ear length or age NaCl - injection. It was also not to suppose from the beginning the positive tendencies of qualitative properties (Coefficient vein visibility).
This investigations were also repeated at a greater random volume.
In the following tables 10 and 11 are calculated correlative connections between some meanful characteristics within the age groups.

Table 10 : Correlation - and regression coefficients between different parameters of test rabbits within the age group 151 -160 days

Connection	F	r	b_x	b_y
body weight trial start- body weight setting in	18	$0,933^{+++}$	$0,773^{+++}$	$1,126^{+++}$
body weight trial start- ear length	18	$0,556^{++}$	$0,007^{++}$	$170,050^{++}$
body weight trial start- age NaCl	18	0,066	0,003	14,559
ear length - vein visibility coefficient	18	$0,276^{+}$	$0,205^{+}$	$0,374^{+}$

Table 11 : Correlation - and regression coefficients between different parameters within the age group 161 - 170 days

Connection	F	r	b_x	b_y
body weight trial start- body weight setting in	25	$0,447^{+++}$	$0,438^{+++}$	$0,457^{+++}$
body weight trial start- ear length	25	$0,457^{+++}$	$0,001^{+++}$	$196,066^{+++}$
body weight trial start- age NaCl	25	0,100	0,009	1,181

Already becoming clear in the comparison of the statistic numbers, meanful connections within the age groups 151 - 160 days and 161 - 170 days are to be seen.

Essential older animals or animals below this age limit (150 days) do not bring any positive or significant correlations.
Especially clear are the correlations body weight at trial start to the coefficient visibility of ear veins and to the start parameter NaCl.
A great part of results in the correlation and regression analysis is significant.

Summary

The aim of the paper was to give information on basic temperature, relevant lines and age specific influences of various rabbit strains.
At Little Chinchilla and Pyteka was proved the tendency to lower normal temperature. In the basic or normal temperature limits were shown significant divergence to papers of KERSTEN and ZAHN (1963) and DRESSEL (1986).
Precious hints result from a sex comparison, in which male rabbits show a lower normal temperature than female rabbits.
In an average comparison of different age groups rabbits with an age of 151 - 160 days could prove the best suppositions concerning the characteristics body weight and ear development, also optimal normal temperature. In the calculation of correlation and regression coefficients could be reached positive and mostly significant results.
The correlation and regression analysis within the strains in the meanful parameters brought positiv coherences.
In general can be established, that the present investigations can be estimated only as preliminary investigations. All this will be repeated with a higher number of randoms.

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Zusammenfassung

Es war das Ziel der vorliegenden Arbeit, Aussagen zu Grundtemperaturen sowie diesbezügliche linien - und altersspezifische Einflüsse bei verschiedenen Kaninchen - linien zu treffen. Bei Kleinchinchilla und Pyteka konnte die Tendenz zu geringeren Normaltemperaturen nachgewiesen werden. In den Grund - bzw. Normaltemperaturbereichen zeigten sich bedeutsame Abweichungen zu Arbeiten von KERSTEN und ZAHN (1983) sowie DRESSEL (1986). An dieser Stelle wird die Forderung nach Verwendung einheitlicher Rassen bzw. Linien im Pyrogentest deutlich.
Wertvolle Hinweise ergaben sich in einem Geschlechterver - gleich, wo männliche Tiere eine geringere Grundtemperatur als weibliche Tiere aufweisen.
In einem Mittelwertsvergleich von verschiedenen Altersgrup - pen konnten die Kaninchen in einem Alter von 151 bis 160 Tagen die günstigsten Voraussetzungen hinsichtlich der Merkmale Körpermasse und Ohrenentwicklung sowie optimalste Grund - temperaturen nachweisen.
Bei der Berechnung von Korrelations - und Regressionskoeffi- zienten könnten zwischen den genannten Parametern innerhalb dieser und der Altersgruppe 161 - 170 Tage sowie 171 - 180 Tage positive und zumeist signifikante Ergebnisse erzielt werden.
Die Korrelations - und Regressionsanalysen innerhalb der einzelnen Linien erbrachten innerhalb der bedeutsamen Merk - male Körpermasse bei Versuchsbeginn, Ohrlänge, Grundtemperatur bei NaCl - Injektion und Alter NaCl - Injektion positive Zusammenhänge. Die Linie Kalifornier empfahl sich dabei hinsichtlich ihrer quantitativen und qualitativen Parameter.

Es ist generell festzustellen, daß die vorliegenden Be - trachtungen nur als Voruntersuchungen zu werten sind, da das Stichprobenmaterial in einigen Fällen zu gering war. Dies konnte jedoch ausreichen, um gewisse Tendenzen in den einzelnen Beziehungen zu erkennen. Alle Untersuchungen werden mit größerer Stichprobe wiederholt.

World Rabbit Science Association