Effects of ketamine anaesthesia, stress and repeated bleeding on the haematology of vervet monkeys

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Summary
Haematology values are presented for the vervet monkey (Cercopithecus aethiops), and the relative effects of high dose ketamine anaesthesia, stress of capture and repeated bleeding assessed. Anaesthesia resulted in decreased WBC and RBC values, attributed to depression of cardiovascular function. These effects were the reverse of those of alarm and strenuous exercise (isocyanide and polycythemia) during capture. Stress resulted in relatively high white and low red blood cell counts. Opposing effects of stress and anaesthesia led to comparable haematological values for trained, non-anesthetized vervets and stressed, anesthetized vervets. Effects of repeated bleedings were opposite in anesthetized and non-anesthetized animals. These effects, however, along with those of ketamine anaesthesia and stress, were relatively insignificant compared with the wide variation in haematological values found among individuals. The biological importance of these effects thus appears to be slight. The concept of "normal values" is discussed.

Ketamine hydrochloride anaesthesia is used routinely by the Institute of Primate Research (IPR) for various treatments and procedures. Its effectiveness and safety as an anaesthetic agent for non-human primates has been reported (Brée, Felder & Couten 1987; Brée, 1987). Although haematology values for monkeys given repeated doses have been reported as remaining within the normal range (McCarthy, Chen, Kassou & Emsri, 1985), significant changes in haematological findings have been noted in rhesus monkeys (Loosli, Hendrickson & Anderson, 1980). Any such effects on the blood picture must be taken into account when assessing "normal values" and when interpreting clinical and experimental data. This report presents a comparison of haematological data between ketamine-anaesthetized and non-anaesthetized vervet monkeys (C. aethiops). Effects of repeated bleedings in the 2 groups is also investigated. Clinicians and researchers are sometimes reluctant to rely on haematology findings in samples from captured animals, on the grounds that stress and exercise may cause changes which obscure clinical interpretation. A third group is thus presented to show the combined effects of stress of capture and ketamine anaesthesia upon the blood picture. Because published haematological findings for this species are few, this study will enhance the body of reference values for the vervet monkey.

Materials and methods

Animals
The study was designed to coincide with other research protocols, resulting in variations in sampling schedules and number of animals in the 3 groups. 32 adult, non-pregnant females (group 1) were anaesthetized with ketamine hydrochloride (Vetalar: Parke Davis & Co., Pettypool, Cowley, Oxford, UK) (30 mg/kg i.m. twice weekly for 2 weeks after overnight fasting. Within 15 min of injection, 1 ml of blood was withdrawn from the femoral vein and placed in a tube containing kaolin and 2.5 mg/mL EDTA. The blood was delivered to the laboratory within 15 min and processed within 1 h of arrival. For comparison, 10 adult, non-pregnant females (group 2) trained to present a blood sample were bled weekly for 6 weeks without anaesthesia. The samples were processed in the same manner as for group 1. Both groups were housed in individual indoor cages and were accustomed to daily handling. Diet consisted of commercial primate chow supplemented with fresh vegetables and fruit; an available water bottle was provided ad libitum. 32 adult females (group 3) housed in a group outdoor cage were sampled once after ketamine anaesthesia. Unlike groups 1 and 2, these animals were not used to being handled and were caught with nets before sampling. Samples were again taken within 15 min of anaesthesia and processed in the above-mentioned manner. Diet was the same as for groups 1 and 2.

Laboratory methods
Red blood cells (RBC) and white blood cells (WBC)
were determined by an electronic cell counter (Coulter Counter II, Coulter Electronics Ltd, Harpenden, Hertfordshire, UK) and haemoglobin (Hb) with a haemoglobinometer (Hematoglobino-

moter, Coulter Electronics Ltd) using the cyan-

methaemoglobin method. Quality control was per-

formed using commercial controls (IC Coulter

Counter Cell Control, Coulter Electronics Ltd).

Hematocrit (PCV) was calculated with a micro-

hematocrit reader (Hawksley Reader, Hawksley &

Son Ltd, Lancing, Sussex, UK). Total protein and

ferritin were read from a refractometer (Atago

SP-N, Atago Co., Ltd, Tokyo, Japan) using the 56
centrifugation method for ferritin (Schoen,

Jan & Carroll, 1975). Mean corpuscular volume
(MCV) mean corpuscular haemoglobin concen-

trations (MCHC) and mean corpuscular haemoglobin

(MCH) were calculated from RBC, Hb, and PCV

values. Blood films were made using the coverslip

method and stained with Wright's. WBC differen-
tials were performed by counting 200 WBCs. Hb,

total protein, and ferritin values were deter-
m ined in commonly used units, and also reported in

SI units.

Statistical analysis

2-way analysis of variance was used to study the

effects of repeated blood sampling and individual

differences among animals in group 2. The analysis

of variance was also used to study the effects of

repeated sampling in group 2 animals, although data

from the 6th bleeding was omitted as some indi-

viduals were only spliced 5 times. Data on eosinophils

and basophils were not included in either analysis,

due to the sporadic occurrence of these cell types.

Initially, in comparing the 3 groups, the average of

all consecutive bleedings was taken for each indi-

vidual of each group 1 and 2. This process some

problems in interpretation, as there were statisti-

cally detectable effects due to the repeated bleeding,

resulting in the blood parameters of each individual

in these groups being estimated with greater preci-

sion than in group 3. Accordingly, the analysis was

also carried out using only the data from the 1st

bleeding in these 2 groups.

As the aim of the experiment was to study the

over-all pattern of response to the three different

bleeding treatments, a principal component analy-

sis (Hinton, Jones & Fasters, 1982) was also used

both to study the pattern of variation separately in

groups 1 and 2, and also in comparing the 3 groups.

Such an analysis attempts to extract components

called "canonical" or "principal components" or some

other factor which depend on a composite pattern of

changes in blood parameters. Individual animals may

then be characterized by these components, and the

results may be displayed graphically. The advantage

of the technique is that in many cases, it can reduce

the dimensionality of the data to that a large

proportion of the total variation may be accounted

for by a few of the named components.

Results

Table 1 shows the mean for each bleeding of the 5

characteristics in which there were statistically signif-

icant \( P < 0.05 \) differences between bleedings in

either group 1 or group 2, or both. In group 1, the

Hb and PCV levels were higher and the total protein

was lower than in the first or in subsequent bleedings.

In contrast, in group 2 the PCV was lower and the

total protein was higher than in subsequent bleedings.

In group 2 the white cells also increased, though in
group 1 they remained the same. Thus, the response of the 2
groups to repeated bleeding differed significantly.

In both groups, the principal component analysis

yielded 2 components which together accounted for

more than 50% of the total variation. A component

"red vs white cells" accounted for 53.3% of the varia-
tion in group 1 and 25% of the variation in group 2.

This had negative correlations with the RBC, Hb

and PCV values, and positive correlations with the

other 6 blood parameters assessed. Thus, a high

score represents low red cell values, high white

cell, total protein, and ferritin. A second component

"blood cellularity" accounted for 25% of the

variation in the first group and 30% in the

second. This had positive correlations with all blood

parameters except total protein. Thus, animals with

a high score had high cell counts and low total

protein.

The scores for these 2 components for each

bleeding are also shown in Table 1. These also

show clearly that the 2 groups responded entirely
differently to repeated bleeding. In group 1 (with

ketamine anaesthesia) the red cells decreased and

the white cells increased from the first to the last

bleeding, whereas in group 2 the opposite occurred,

apart from the last bleeding. A similar picture

emerged with respect to blood cellularity. In group 1

it is decreased with repeated bleeds, whereas in

group 2 it is increased.

2-way analysis of variance of these 2 components

was used in order to quantify the relative impor-
tance of differences among individuals, bleeding

times and experimental error. For all characters,

there were large and highly significant differences

among individual animals in both groups 1 and 2.

Variance component analysis was used to measure

the relative importance of these 3 factors. The

results are shown in Table 2. In the case of the "red

vs white cell" component, although statistically
| Character | Bleeding
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>number</td>
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<td>1</td>
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<td></td>
<td>2</td>
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<td></td>
<td>3</td>
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<td>4</td>
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<td></td>
<td>5</td>
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<tr>
<td>PCV</td>
<td>1</td>
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<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3</td>
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<tr>
<td></td>
<td>4</td>
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<tr>
<td></td>
<td>5</td>
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<tr>
<td>Lymphocytes</td>
<td>1</td>
</tr>
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<td></td>
<td>2</td>
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<tr>
<td></td>
<td>3</td>
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<td>4</td>
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<td>Total protein</td>
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<tr>
<td></td>
<td>3</td>
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<tr>
<td></td>
<td>4</td>
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<tr>
<td></td>
<td>5</td>
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<tr>
<td>Red vs. white cell ratio</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
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<td>2</td>
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<td></td>
<td>3</td>
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<tr>
<td></td>
<td>4</td>
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<td></td>
<td>5</td>
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</table>

Highly significant differences were detected between the different bleedings, the effects were extremely small in comparison with individual differences, which generally accounted for 70% or more of the total variation. The effect was not as clear with the cost of blood cellularity, where in group 1 the individual differences were not so marked as in group 2, where again differences between bleedings accounted for a relatively trivial proportion (less than 5%) of the total variation. Thus, in 3 out of 8 analyses, although the effect of repeated bleeding was statistically significant, it was relatively unimportant compared with individual differences and in all cases it was of the same order of magnitude as the experimental error (i.e., the

Table 2. Variance components from 2-way analysis of variance of principal components scores.

<table>
<thead>
<tr>
<th>Character</th>
<th>R1</th>
<th>R2</th>
<th>B1</th>
<th>B2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>9.9</td>
<td>1.9</td>
<td>1.9</td>
<td>7.4</td>
</tr>
<tr>
<td>Between individuals</td>
<td>5.5</td>
<td>1.9</td>
<td>3.2</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Experimental error
interaction between bleeding and individual differences.

In comparing the 3 groups based on the mean
swim across all bleedings in groups 1 and 2, the
analysis of variance showed small but statistically
significant differences among groups for hematoc-

ion, WBC, neutrophils, lymphocytes, monocytes,
total protein and fibrinogen. The means are shown
in Table 3. The principal components analysis again
resulted in a ‘red vs white cell’ factor accounting for
31%, and a ‘blood cellularity’ factor accounting for
27% of the total variation. Differences among
the groups were highly significant (P < 0.01) for the
first principal component, and were significant at the 5%,
level for the second.

Fig. 1 shows graphically individuated of each of
the 3 groups in relation to the first and second principal
components. The circles represent 2 standard devia-
tions from the mean (averaging the standard devia-
tions for group 1 and group 2, and thus would be
expected to contain approximately 65% of all
individuals. On the first PC, groups 1 and 2 (both
unanaesthetized apart from any stress resulting from
repeated bleeding but differing in that group 1 was
anaesthetized) do not differ, but group 3 (anaesthetized
but unanaesthetized) differs from both of them. On the
second PC, groups 2 and 3 do not differ, but group 2
differs significantly from both of these. Note that
ketamine reduced blood cellularity (i.e. red and
white blood cell counts), and stress (group 3)
resulted in relatively high white and low red blood
cell counts. Note also that the differences among
groups appear to be relatively small in comparison
with individual variation within each group.

Finally, the data were re-analysed using only the
data from the first bleeding in groups 1 and 2. The
results were slightly different in this case in that
the first principal component, accounting for 33% of
the total variation, was interpretable as a ‘white cell
response’, correlated with the red blood cell
values, but correlated with all the white cell
parameters, total protein and fibrinogen. Group 1
had a significantly lower white cell score than the
other 2 groups, which did not differ from each other.
The second principal component was interpretable as
a ‘red cell response’, but was only accounted for
17% of the variation and did not differ significantly
among groups.

**Discussion**

The haematological reaction to stress, termed the
‘alarm reaction’, characterized haematologically by
leucocytosis and haemoconcentration, has been
shown to be attenuated in rhesus monkeys habitu-
ated to sampling procedures (Hicks & Dack, 1966). It
has been postulated that ketamine may also act to
reverse the ‘alarm reaction’ by attenuation of the
initial stress response subsequent to central adminis-

Although ketamine has unusual pressor effects
(increased blood pressure, respiratory rate and
heart rate) at lower dose levels (2-5 mg/kg)
(Sawyer, 1975), higher dosages (10-20 mg/kg) are
attenuated by depressor effects more similar to those
induced by other anaesthetic agents (Booth, Good-
neighbor, & Bush, 1970; Oehme, 1977; Parker &
the higher dose level used here (10 mg/kg), de-
pressed cardiovascular function in the unanaesthetized
animals is expected. Cardiovascular function in
these monogonads has been noted in these
monkeys (Loomis, Hentz & Anderson, 1960)
as well as in other species under other types of
anaesthesia (Kracov, 1974). The ‘white cell responses’ noted in group 1 but not in group 3
suggests that the added component of stress serves
to counteract the lowering of white cell counts
in anaesthetized versus

The increase in numbers of monocytes in group 3
may be attributable to stress release provoked by
stress. Such an effect has been reported in dogs
injected with ACTH (Selmin, Jain & Carol, 1975).
In that study, eosinophilia was also observed, but
was not marked until 4 h after ACTH injection,
whereas mild mononucleosis was noted in the first
(2 h) postinjection sample. This clarifies the absence
of neutrophilic or monocytes in group 3, which was
sampled shortly after capture and anesthesia.

While depressed cardiovascular function induced by
high dose ketamine anesthesia may explain the
low WBC and PCV values of group 1, it is
consistent with the elevation of serum protein
values in this group. Haemorrhage, rather than
haemoconcentration, would be expected in this
case. The differences in group means are not great
(7-9, 7-6 and 7-1 g/dl in groups 1, 2 and 3), and their
import is unclear. Fibrinogen levels also varied
among groups, but differences among group
means were lower than machine precision levels, it
is doubtful if these are meaningful.

Experimental protocols often call for repeated
bleeding of laboratory animals; a haematological
response to such repetition could affect interpreta-
tion of both research and clinical data. The
apparently opposite responses exhibited by groups 1
and 3 suggest that repeated bleeding with ketamine
anaesthesia is initially not stressful but becomes so.
Table 3. Hematology findings on ketamine.

<table>
<thead>
<tr>
<th></th>
<th>RBC (x 10^6/µL)</th>
<th>Hb (g/dL)</th>
<th>PCV (%)</th>
<th>MCV (fl)</th>
<th>MCHC (g/dL)</th>
<th>MCH (pg)</th>
<th>Total protein (g/dL)</th>
<th>Fibre (mg/l)</th>
</tr>
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<tbody>
<tr>
<td><strong>Group 1</strong></td>
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</tr>
<tr>
<td>non-stressed</td>
<td>Mean 5.28</td>
<td>13.5</td>
<td>10.5</td>
<td>75</td>
<td>30.3</td>
<td>25.7</td>
<td>79</td>
<td>3500</td>
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<tr>
<td></td>
<td>Unstressed SD</td>
<td>0.71</td>
<td>1.7</td>
<td>1.5</td>
<td>0.8</td>
<td>0.5</td>
<td>9</td>
<td>1900</td>
</tr>
<tr>
<td>(12 animals)</td>
<td>48 determinations</td>
<td>Range 3.36-6.72</td>
<td>9.5-16.1</td>
<td>5-181</td>
<td>88-88</td>
<td>31-4</td>
<td>26-3</td>
<td>8800-8000</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>non-stressed</td>
<td>Mean 5.00</td>
<td>12.0</td>
<td>9.5</td>
<td>76</td>
<td>29.8</td>
<td>24.5</td>
<td>75</td>
<td>2400</td>
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<tr>
<td></td>
<td>Unstressed SD</td>
<td>0.49</td>
<td>1.2</td>
<td>1.2</td>
<td>0.8</td>
<td>0.2</td>
<td>0.6</td>
<td>1090</td>
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<tr>
<td>(12 animals)</td>
<td>48 determinations</td>
<td>Range 4.53-6.88</td>
<td>12.5-17.6</td>
<td>9-178</td>
<td>88-88</td>
<td>26-3</td>
<td>22-2</td>
<td>6500-6000</td>
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<tr>
<td><strong>Group 3</strong></td>
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<td></td>
<td></td>
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<tr>
<td>stressed</td>
<td>Mean 5.32</td>
<td>12.9</td>
<td>10.9</td>
<td>73</td>
<td>35.1</td>
<td>25.4</td>
<td>71</td>
<td>4000</td>
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<td></td>
<td>Unstressed SD</td>
<td>0.96</td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
<td>1.9</td>
<td>0.9</td>
<td>2000</td>
</tr>
<tr>
<td>(12 animals)</td>
<td>48 determinations</td>
<td>Range 4.04-6.45</td>
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<td>7-122</td>
<td>88-88</td>
<td>26-3</td>
<td>22-2</td>
<td>6000-6000</td>
</tr>
</tbody>
</table>

Fig. 5: Scatter plot of red vs white blood cells against 'blood cellularity' in the 3 groups of animals. Circles drawn at 2 standard deviations (arrows) between the 2 characters from the mean, thus should encompass about 95% of individuals.
Vervet haematology

whereas repeated bleeding in tame animals without anaesthesia is initially stressful, but becomes less so with each bleeding.

The various effects of stress, ketamine anaesthesia, and repeated bleedings may appear to make interpretation of haematology values difficult, results of this study show that such effects are relatively insignificant when compared with the wide range of values found among individuals in all three groups. Even wide individual variation has been reported for other non-human primate species (Schulte, Jan & Carroll, 1977). Thus, the biological significance of these haematological responses appears to be slight, though they would depend on the purpose for which the data are being collected.

The concept of 'normal' comes into question. It would be difficult to ascertain which of the 3 groups studied here or which of the bleedings could be considered most 'normal'. Considering the great variation seen among individuals with this problem in defining normality, it becomes apparent that published reference values, while useful to a degree, should be viewed with a certain restraint. Ideally, individual baseline values should be established for both clinical and research use.

References


Die Auswirkungen von Ketaminanästhesie, Stress und wiederholten Blutungen auf die Hämatologie von Meerkatzen

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Zusammenfassung