What a lot is concealed in one drop of blood!

- Experimental Dark-field Microscopy Investigations of Living Blood

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Editorial preface:

As part of a term paper for a high level Biology course at the Rabanus-Maurus Gymnasium [= Grammar school] in Mainz, Ms. Fiedermutz tackled the subject „Experimental Investigations of Living Blood as seen through the Dark-field Microscope". She investigated the influence of foodstuffs and nicotine on the blood, using Dark-field microscopy to illustrate her findings. We reprint her paper here in an abbreviated form.

1. Basic Principles

1.1 Tasks of the Blood

The blood's most important task is to transport various substances. In this way water and nutrients pass from the organs of digestion and storage via the blood to the tissues, by-products reach the organs of elimination, and hormones and other messenger substances arrive at their response organs or cells. Within the organism oxygen and carbon dioxide too are distributed via the blood from the respiratory organs to the tissues, or vice-versa. Temperature regulation likewise takes place with the help of the blood, since warmth reaches the body's surface, from where it is emitted.

Functions of the blood itself include clotting, which serves to close wounds, and also its buffering capacity, which guarantees a constant chemical milieu so far as pH level and ionic balance are concerned.

As well as this, the blood has a defensive function. This includes antibody formation and phagocytosis, affording protection against pathogenic microbes, toxins or foreign substances.

1.2 Haemorrheological factors under the influence of cigarette smoke.

Various studies support the view that smoking cigarettes has an adverse effect on the fluid properties and clotting of the blood (Belch 1984; Daibianco 1989; Feher 1990; Gudmundsson & Bjelle 1993).

Cigarette smoke consists mostly of carbon monoxide (CO), and when inhaled, this has a much greater affinity for haemoglobin than oxygen (O_2). The result of this reduced capacity for oxygen bonding is a reduction in the oxygen supply to the tissues. Because of this hypoxia in the tissues, the brain signals the bone marrow to produce more red blood-cells.

This results in a higher level of carboxyhaemoglobin (CO bonding with haemoglobin) in the blood, and Sagone regards this as being the cause of the hypoxia; Anadere on the other hand suspects an increased aggregation of erythrocytes to be the reason.

As a consequence there is a rise in the haematocrit - the amount of the cellular component - and a thickening of the blood occurs. This is the reason for the deterioration in the viscosity (fluidity) of the blood. Moreover, the red blood-cells are limited as to their formability - and thus their distortability (Leonhardt), and this places a burden on the supply of O_2 (Salbas, 1994).
The viscosity of the blood is influenced by its water content, the number of erythrocytes, their formability, the haematocrit level (see Fig. 1), the temperature and, to a lesser extent, by the amount of plasma protein.

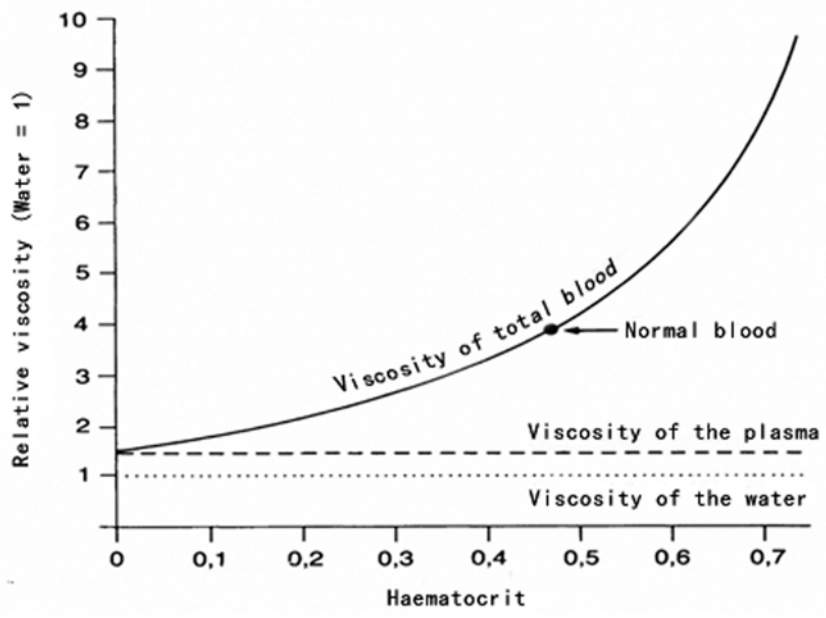


Fig.1: Dependency of the relative viscosity of the blood on the haematocrit level, after Guyton

In smokers the viscosity of the blood is elevated.

One particular property of erythrocytes is their clustering together like a roll of coins (rouleaux formation or pseudo-agglutination), which is easily observed with a dark-field microscope.

As the viscosity increases, so also does the quantity of leucocytes (Blann 1992; Calori 1996; Thomas 1996) as well as the tendency of the thrombocytes to aggregate (Pittilo 1984) i.e. thrombi develop, since the platelets become stickier and stickier. The plasma viscosity increases, not only on account of the formation of aggregates, but also because of the elevated fibrinogen which is caused by smoking. The long-chain protein molecule is capable of building bridges. Thus, because of its bonding with the erythrocytes, protein can have a negative effect on the viscosity.

Viewed through the dark-field microscope, it is possible to demonstrate - as well as the above-mentioned aggregation of thrombocytes - the aggravated viscosity in the form of network-like threads of fibrin. The transformation of fibrinogen into fibrin, the active form, points to increased activity of the clotting factors, which makes the blood lumpy.

1.3 Digestive Leucocytosis

By the term 'digestive leucocytosis' we understand an increase in the leucocyte count after consuming food. This was first discovered by Rudolf Virchow in the 19th century. It is not yet known to what extent this constitutes an immune response to foreign protein in the food, or a physiological reaction to a high amount of fat.

After food with a high protein content, e.g. after eating meat, digestive leucocytosis is significantly higher than it is after eating raw food. Thus it follows that the fluid properties of the blood are better in someone on a raw food diet, eating food in its natural state, i.e. unprocessed.

By means of investigations of the blood with the aid of a dark-field microscope, I have attempted to demonstrate the influences of cigarette smoking and the type of diet, as described above.

1.4.1 Investigations of Living Blood with the Aid of Dark-field Microscopy

Dark-field microscopy makes it possible to investigate transparent objects and those lacking in contrast without prior staining. Living objects can likewise be observed.

Among the solid components of the blood, it is possible to distinguish between erythrocytes, thrombocytes, leucocytes and lymphocytes. Brownian movement of the protein particles, arising from the blood's own warmth is likewise visible under the dark-field microscope.

1.4.2. The Principle of a Dark-field Microscope

A simple example from nature will help us easily to understand the principle of dark-field microscopy. Viewed in front of a light background, spiders' webs are barely visible. However, if one views them from a different angle, so that the delicate structures are lit from the side with a dark background, then the spiders' webs really appear to light up.

Using this example, the phenomenon of structures lighting up against a dark background can well be applied to the way in which a dark-field microscope works.

2. The Experiments

2.1. The Procedure Followed in Investigating Blood

2.1.1. Experiment 1: Comparison of Smokers and Non-smokers

Four smokers and four non-smokers were available for this series of investigations.

I took fasting blood samples from the latter in the mornings. These were for the purpose of comparison. Water was the only drink permitted.

Initially fasting blood samples were likewise taken from the smokers at 8:00 in the morning. Immediately after that they smoked one cigarette. After a wait of 15 minutes the second blood sample was taken. Around 17:00 hours a

final blood sample was taken, after varying numbers of cigarettes had been smoked. The subjects were asked to keep a count of all the cigarettes they had smoked between 08:15 and 17:00 hrs.

2.1.2. Experiment 2: Dietary Comparison of Raw Fruit and Vegetables Vis-à-vis Meat.

Three non-smokers carried out this experiment, so as to avoid any distortion of the results that might be caused by smoking.

Firstly, at 8:00 a.m., fasting blood samples were taken for purposes of comparison.

For their raw food day the subjects had raw fruit, such as apples, pears, mandarins, oranges and bananas for breakfast; then, for lunch, a salad of tomato, cucumber and carrot. As well as water, tea and juice were permitted drinks. At 16:00 hrs. in the afternoon, the blood samples were taken.

On another day meat consumption was tested. In the morning there was chicken soup; in the middle of the day half a grilled chicken without any side dishes. By way of comparison one of the subjects ate pork.

Here again, after lunch and a wait of one hour, a blood sample was taken.
The samples were examined under the dark-field microscope and compared using digital photography.

Both experiments were carried out three times, so as to achieve a conclusion which was statistical to some extent.

Smoking Experiment:			
Subject	**Cigarettes**	**Run**	**Dark-field Findings**
E	1	First Second Third	Coin-rolls (rouleaux) Aggregation of thrombocytes Fibrin + aggregation of thrombocytes
	8-10	First Second Third	Leucocytosis Fibrin (slight) + aggregation of thrombocytes Coin-rolls (rouleaux)
F	1	First Second Third	Leucocytosis + fibrin Fibrin + leucocytosis Fibrin + slight leucocytosis
	4-7	First Second Third	Fibrin + slight leucocytosis Fibrin Fibrin + leucocytosis
G	1	First Second Third	Fibrin Fibrin Fibrin
	7-9	First Second Third	Coin-rolls (rouleaux) Fibrin Coin-rolls (rouleaux)
H	1	First Second Third	Coin-rolls (extreme) Coin-rolls (extreme) Coin-rolls + fibrin (slightly between them)
	30-36	First Second Third	Aggregation of thrombocytes Coin-rolls + fibrin (slight) Fibrin

Table 1: Summary of dark-field investigations in smokers

2.2. Results of the Blood Investigations and Their Interpretation

2.2.1. Experiment 1: Influence of Cigarette Smoking

In all four test subjects (E, F, G and H) the fibrin level increased significantly **after smoking one cigarette,** compared with the fasting state. In two of the smokers multiple coin-rolls (rouleaux) developed, and in one subject the leucocyte count increased. In one smoker aggregation of thrombocytes also occurred.

Following the varied consumption of several cigarettes, compared with the fasting state, an elevated fibrin level could be observed in all of them. In three subjects coin-rolls (rouleaux) occurred. In two smokers leucocytosis

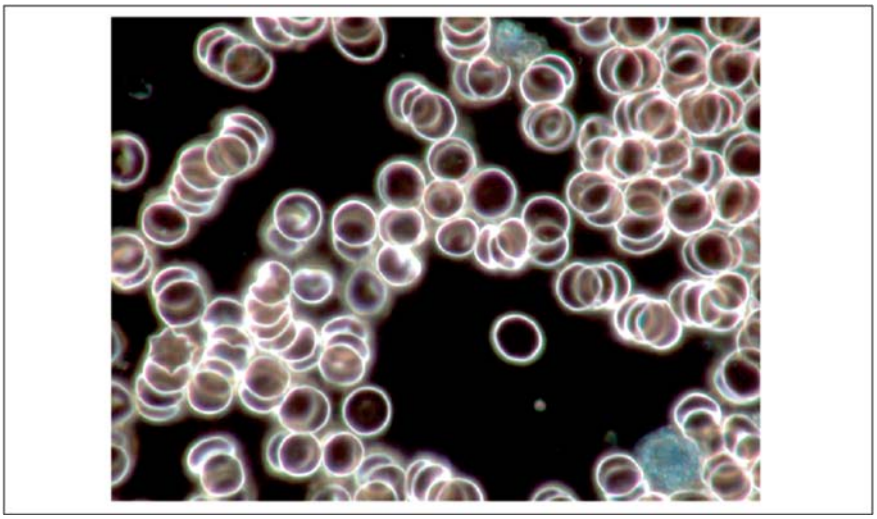
and multiple aggregation of thrombocytes were established. The precise information is shown in Table 1.

There are no observable differences between non-smokers and smokers after fasting. However, the blood of the test subjects following cigarette smoking showed a considerably worse fluidity.

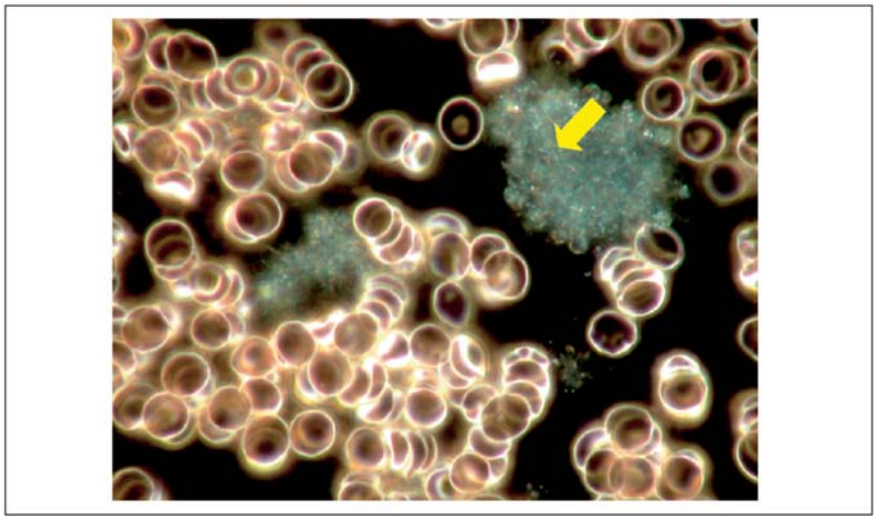
The percentage values in my statistical diagrams are obtained from the frequencies of all the observations together (see Fig. 2).

In 47.06% an elevated fibrin content occurred after smoking just one cigarette, in 23.53% increased Rouleaux formation occurred, in 17.65% leucocytosis, and in 11.76% there was aggregation of thrombocytes. Similar results were observed after the consumption of several cigarettes. The viscosity of the blood increased. It was not possible to test for the haemocrit level.

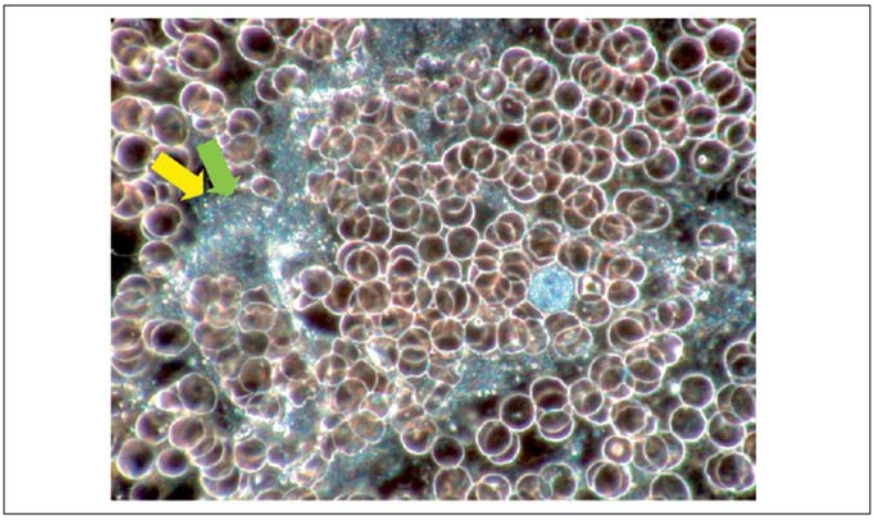
These findings suggest an obstruction to the oxygen transport in the body. The blood cell aggregations may be regarded as tiny thrombi which are capable of blocking small blood vessels.
It is scientifically proven that there is an increased risk of cardiovascular disease, such as strokes and cardiac infarction, in those who inhale smoke over many years.



Picture 1: Test subject E, smoker, after fasting; (enlargement x 1000) 20.01.08



Picture 2: Test subject E, smoker, after 1 cigarette, Run 2; (enlargement x 1000), 02.02.08; showing aggregation of thrombocytes



Picture 3: Test subject E, smoker, after 8 cigarettes, Run 2; (enlargement x 1000), 01.03.08; (green arrow shows fibrin; yellow arrow shows aggregation of thrombocytes)

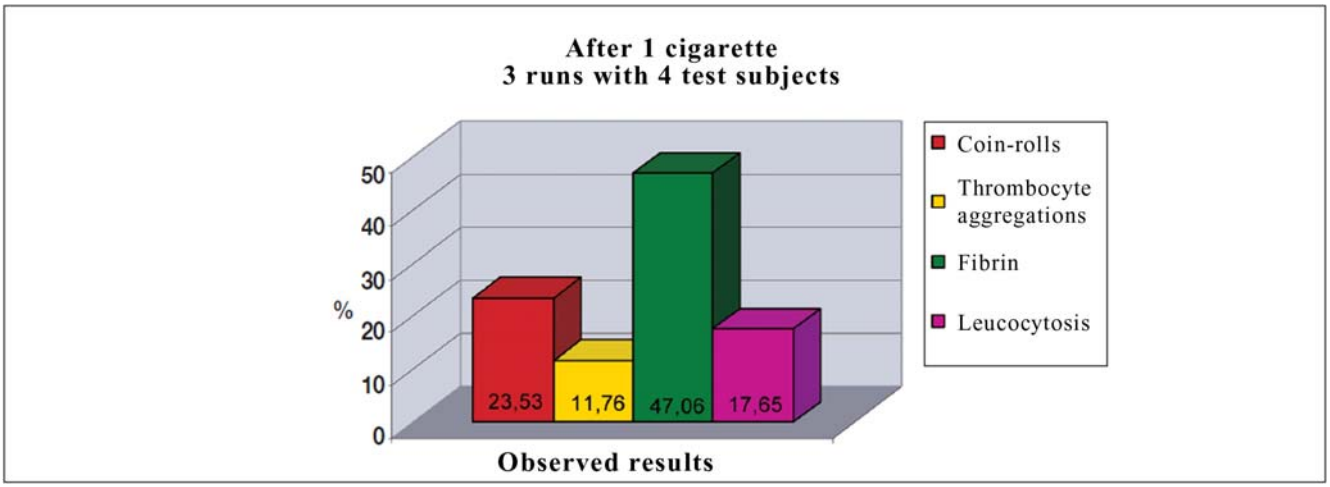


Fig. 2: Percentage frequency of changes in the blood after cigarette consumption

Dietary Experiment - Consumption of Meat Test Subject and Test Run.		**Dark-field Findings**
		Summary of findings • Poor fluidity (erythrocytes deformed and agglomerated) • Aggregation of thrombocytes • Leucocytosis • Coin-rolls (rouleaux) • Fibrin
Test subject A	Run 1 Run 2 Run 3	Leucocytosis + coin-rolls (rouleaux) Coin-rolls (rouleaux) Leucocytosis + coin-rolls
Test subject B	Run 1 Run 2 Run 3	Leucocytosis Leucocytosis + fibrin Leucocytosis
Test subject C	Run 1 Run 2 Run 3	Leucocytosis Leucocytosis Leucocytosis + fibrin + thrombocyte aggregation

Table 2: Summary of dark-field investigations for meat consumption

Dietary Experiment - Raw Food Consumption Test Subject and Test Run		**Dark-field Findings**
Test subject A	Run 1 Run 2 Run 3	Good fluidity (large intervals between circular erythrocytes) No aggregations Few leucocytes No coin-rolls (rouleaux) No fibrin
Test subject B	Run 1 Run 2 Run 3	Findings as for Test subject A
Test subject C	Run 1 Run 2 Run 3	Findings as for Test subject A

Table 3: Summary of dark-field investigations for consumption of raw fruit and vegetables

2.2.2. Experiment 2: Dietary Influence

Differences in the fluidity of the blood between test subjects (A, B and C) could also be established in the series of tests regarding dietary influence.

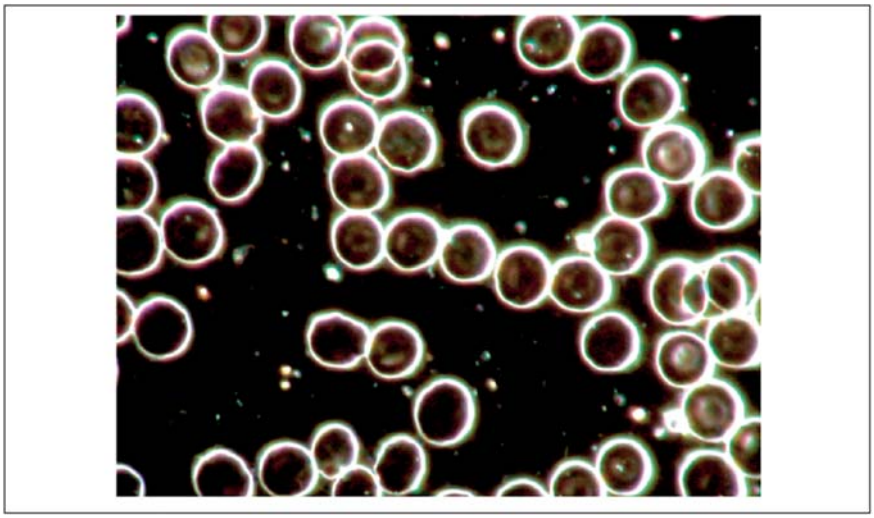
The fluidity was substantially better following consumption of raw fruit and vegetables than after meat consumption. In all the test subjects and in every run of the test, those who consumed raw food exhibited no increased aggregations, no increased formation of coin-rolls or fibrin and, in particular, few leucocytes. The erythrocytes were not deformed. In most cases the plasma was particularly clean (see Table 3).

On the contrary, digestive leucocytosis could be observed in all the test subjects **after consumption of meat**, although this only became visible at/beyond a magnification of 400x. This was confirmed when the test was repeated.

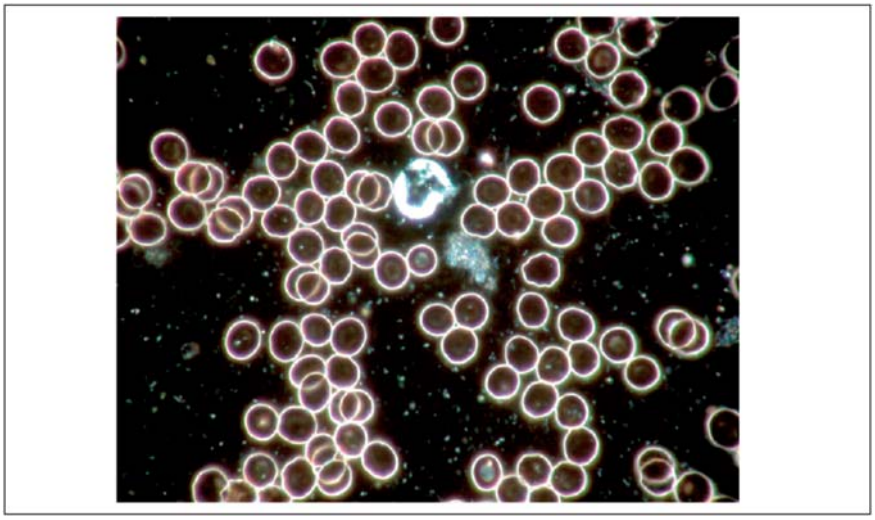
As well as this, one person showed a tendency to coin-rolls (rouleaux), two to increased fibrin and one of the three subjects to aggregation of thrombocytes (see Table 2).

For purposes of comparison, refer to the photographs with coloured arrows pointing to corresponding structures, and to the statistical diagrams.

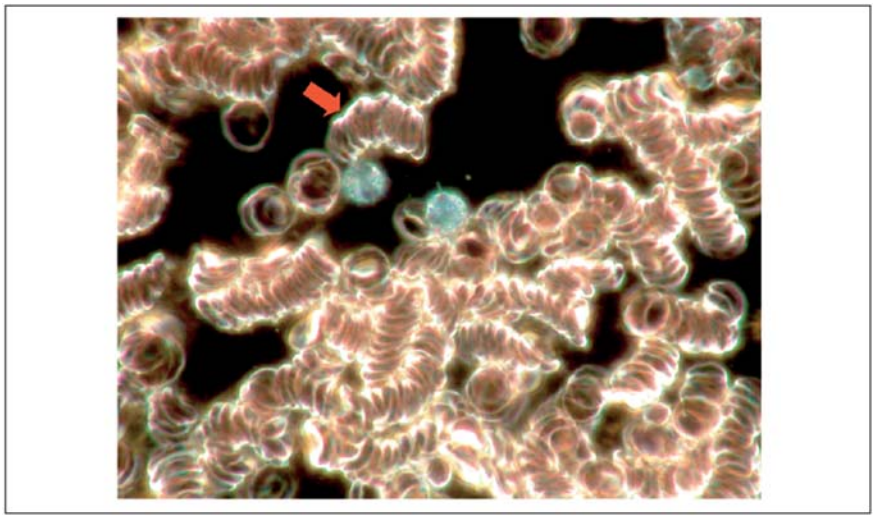
Following consumption of meat, the subjects' blood exhibited a markedly worse fluidity.



Picture 4: Test subject A, non-smoker, after fasting; (enlargement x 1000) 27.01.08



Picture 5: Test subject A, non-smoker, after meal of raw food, Run 1; (enlargement x 1000), 27.01.08



Picture 6: Test subject A, non-smoker, after eating meat, Run 1; (enlargement x 1000), 09.02.08; (red arrow shows rouleaux formation)

The percentage values in my statistical diagrams are obtained from the frequencies of all the observations together.

As can be seen from Fig. 3, digestive leucocytosis occurred in 57.14% of the test subjects following meat consumption, as compared with raw food consumption. Rouleaux formation occurred in 21.43%, elevated fibrin content in 14.29%, and in 7.14% aggregation of thrombocytes. None of these phenomena was observed on any occasion whatsoever after consumption of raw fruit and vegetables. Moreover the blood plasma appeared very clear, unlike that of the meat-eaters.

Thus, in my estimation, the bloodflow was significantly better in those who ate raw food than in the meat- eaters.

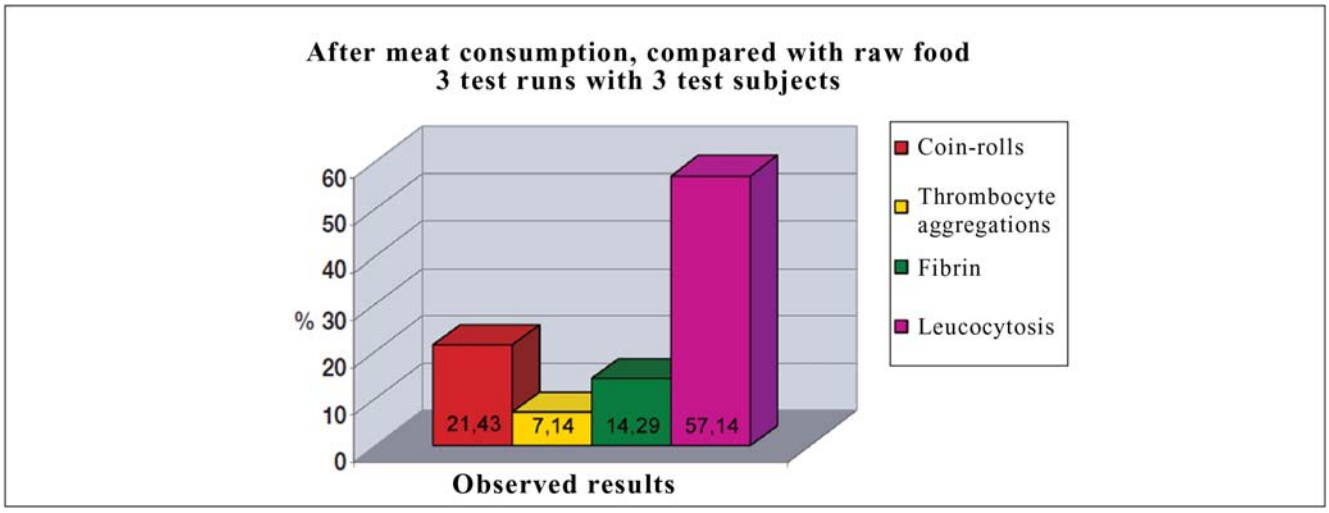


Fig.3: Percentage frequency of changes in blood after meat consumption

3. Consideration of Errors in Carrying out the Experiments

It is highly possible that the experimental results could be influenced by such factors as age, height, weight - overweight, underweight - menstruation and use of the contraceptive pill. In order to evaluate this it would be necessary to carry out larger series of experiments, distinguishing between the above-mentioned criteria. Moreover, in the smoking/ non-smoking groups, again it is possible that substantial changes could be brought about by variations in diet.

4. Summary

One aim of this study was to ascertain the influence of cigarette smoking on the blood, so as to draw conclusions regarding pathological risks.

The investigation confirmed that a considerable deterioration in the fluid properties of the blood takes place as a result of increased rouleaux formation and aggregation of thrombocytes, leucocytosis and augmented fibrin formation. This results in an increased risk of diseases such as, for instance, cardiac infarction and strokes, owing to obstructed oxygen transport.

In the second part, where a comparison was made in the diet between raw fruit/vegetables and meat, in the latter a deterioration was apparent in the fluidity of the blood, particularly on account of leucocytosis, but also because of rouleaux and fibrin formation, plus aggregation of thrombocytes.

None of these phenomena was observed after raw food had been consumed.

This should not force us to the conclusion that raw fruit and vegetables is the healthier choice of food. The purpose is simply to point up the differences between these two unbalanced dietary styles. In my view, only a balanced diet is really healthy!

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