The scent of fear

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Abstract

In this study we tried to find out if fear can be detected from human body odours. Female subjects wore under-arm axillary pads while watching a terrifying film. Saliva cortisol samples were taken before and after the film presentation as a hormonal measure for the fear response. The fear experience itself was measured by Spielberger’s State-Trait Anxiety Inventory. A “neutral” film, shown one day after the “fear” film, was used as a control in a repeated measures design. In part two of the experiment, the axillary pads were presented to female subjects in a triple forced choice test. Results show that subjects were able to discriminate between fear and non-fear axillary pads, suggesting that women are indeed able to detect “the scent of fear”. A direct correlation between induced fear, changes in cortisol levels and smell ratings could not be established. Thus cortisol levels are probably not the inducer of the scent of fear and a hypothetical fear pheromone could have other origins.

People have always used scents to either mask their body odour or to express and emphasize their moods or appearance. The possibility that odours can also provide relevant biological information about their “sender” has only in recent years become the focus of scientific attention. Doty [1] described the advantages of olfactory communication as follows: the sense of smell even works if the other two “major” senses (visual and acoustic) are functionally restricted (for example if it is too dark or too loud). Moreover, odourous substances can easily be spread over large areas and can last for a long time. Intensity, distribution, and quality of scent marks can give information about size, reproductive and nutritional status, etc. of an individual without immediately drawing unwanted attention to the sender (e.g. detection from a predator via the loud noise, etc. of a sender). Another advantage in communication through odours lies in the fact that sender and receiver don’t have to be in close spatial distance in order to communicate.
The question then becomes prominent: if olfactory communication is useful in other animals, why is it not used by human as well?

In recent years this assumption has undergone major revision. Several studies indicate that humans do indeed seem to use olfactory communication and are able to produce and perceive certain pheromones (for a detailed overview see [2]). Up to now most studies were looking at human pheromones linked to the sex hormones, the question of whether humans are or are not able to communicate other than sex-related information through odours has yet to be raised.

Fear arises in stressful situations that are subjectively perceived as threatening. The intensity of the induced negative feeling corresponds to the subjective perception of threat in a situation. If the negative feeling becomes too intense, a seeking-reaction for stress-relieving mechanisms is initiated [3].

Fear can be induced by external, objective threats (e.g. predators), as well as by internal, subjective threats, called “free floating anxieties” [4]. Free floating anxieties can be generated by conscious or subconscious memories of threatening experiences in the past, or by the mere anticipation of a stressful situation.

The assumption that fear is a learned avoidance reaction to potentially dangerous situations is gradually being questioned. Recent studies show that fear may be a genetically determined function of the nervous system [4]. This hypothesis receives support from an evolutionary point of view. The ability to detect and anticipate dangerous situations seems to be crucial for survival, and individual learning might not be entirely quick enough to ensure survival chances. Moreover, even potentially dangerous stimuli might be rare and thus impossible to learn – leading an individual into danger when the stimulus is encountered for the first time.

Panksepp [4] describes a “major fear circuit” in the brain located within the lateral and central parts of the amygdala in the lobus temporalis, the periaqueductual grey (PAG) of the diencephalon and mesencephalon, and as “output-generating” parts the brain stem and the medulla.

Fear leads to reactions that are both behavioral and physiological. Behavioral reactions include either a “freeze”, “fight”, or “flight” response. Underlying physiological processes include an increase in heart rate, muscular tension, sweating, etc., and, most important, a response of the adrenal gland via the pituitary-hypothalamus-adrenal-axis (HHN) which leads to the release of cortisol. The cortisol level rises as a consequence of the production of corticotropin releasing hormone and ACTH [5]. Thus in many experiments the assessment of changes in cortisol levels is used as an indicator of potentially experienced fear. In a study by Hubert & de Jong-Meyer [6] male subjects showed an increase in cortisol levels (measured from saliva) while they were watching a horror film. Kirschbaum & Hellhammer [7] found similar results. In contrast, Hubert, Möller, & de Jong-Meyer [8] showed that subjects experienced an increase in cortisol levels in response to a funny movie. The authors speculate that every kind of affective arousal and change of mood, positive as well as negative, could be linked to cortisol secretion.

Besides fear inducing situations, everyday stressors, the so-called “daily hassles”, can also provoke a raise in cortisol levels [9; 10]. In fact, it seems that the mere anticipation of a stressful experience can have this effect [11].

It thus remains doubtful that cortisol secretions might function solely as a result of experiencing fear. Cortisol raises in such situations could also be due to the stressing effects of fear.

Alarm pheromones were found in fish as early as 1941 by Von Frisch [12] and have since then been found in many species. Today we know that at least insects, annelids, and fish use olfactory signals to inform their conspecifics about stress, alarm, and fear. In ants, for example, alarm substances can cause aggregation, dispersion, or defense of the colony depending on the sender’s status [13]. The use of alarm pheromones has also been demonstrated in bees (Apis mellifera) [14] and lice (Acrithosiphon pisum) [15]. On “defective stimulation” earthworms (Lumbricus terrestris) give off a substance that makes their conspecifics avoid their area and thus potential predators [16]. Among vertebrates, fish are the best known example for the use of alarm substances: fathead minnows (Pimephales promelas) that have never before been confronted with a pike (Esox lucius) immediately “know” that it is a dangerous predator because the pike seems to get marked by an “odour label” by every minnow it actually catches [17]. Sources of alarm substances in minnows are for example urine, feces, and mucus.

Mammals also seem to use olfactory alarm signals. Valenta & Rigby [18] were able to show that rats can distinguish between the odour of stressed and “relaxed” conspecifics. Carr, Martorano & Krames [19] found that male mice prefer the smell of conspecifics that have just won a fight over the smell conspecifics that have not won a fight, and the smell of conspecifics that haven’t been experimentally shocked, over the smell of conspecifics that have been treated with electric shocks.

In predator-prey-interactions between Mongolian jirds (Meriones unguiculatus) and cats it was shown that the stressed mice mark the “dangerous areas” with their scent and thus tell their conspecifics to avoid these areas, while cats orientated themselves by the scent of stressed mice in order to find them [20].

If other mammals are able to warn their conspecifics – or at least send out the information that they are in a frightening situation – by emitting odourous substances, the question becomes prominent: Do humans possess similar mechanisms? If so, how are these...
mechanisms related to cortisol release? The current research aims to address these questions.

**Methods**

**Collecting the odour samples**

In the first part of the experiment odour samples for later assessment were gathered. In order to do so, rectangular pads (6 x 10 x 1 cm in size) of sterile cellulose were treated with a solution of 40 g Cetaceum in 800 ml diethyl ether. Cetaceum was used because it is odourless and is an ideal fixans for absorbing odours. The pads were then glued to fleece material with pieces of tin foil in between and stored in airtight, odourless plastic bags.

Forty-two female test subjects, aged between 18 and 33 years (mean age: 23.39 S.D. = 3.55) watched a horror film (“Candyman”, © Tristar Pictures Inc., 1992) for 70 minutes and a “neutral” film (“Lokorama”, © S. R. Film & Video Filmproduktions Ges.m.b.H., 1990) – on 2 consecutive days, each presented at the same time of day. The neutral film showed the view from a driver’s seat of a southern-bound train ride, originating in Vienna. The duration was the same as the horror film. While watching the films, participants wore the pads under their armpits to gather the odour samples. Participants had been told to avoid perfumes and deodorants on the days of the experiment and to refrain from smoking and eating odourous dishes. Just before the experiment participants washed their armpits with odourless medical soap and put on cotton t-shirts that had been washed with an odourless detergent and stored in an airtight plastic bag.

Room temperature was kept constant (at 26°Celsius) throughout the experiment. After the experiment, the axillary pads were deep-frozen at −20°C for later assessment.

Before and after the film presentations, participants filled out questionnaires and donated saliva samples with “salivettes” (Fa. Sarstedt, Rommelsdorf/Germany). The salivettes then were deep-frozen at −20°C Celsius.

The questionnaire consisted of three parts: in the first part the women were asked if they had shaved their armpits, if they were taking oral contraceptives, their mean cycle length, day of the cycle, and if they were on any medication. The second part of the questionnaire consisted of Spielberger’s [3] State-Trait-Anxiety Inventory (STAI). In the third part, participants were asked to rate the film they had just been watching on a 1–10 scale by the adjectives: a) boring, b) frightening, c) suspenseful, and d) funny. The state anxiety scores were later used to check if the actual situation had evoked fear in the subjects; trait anxiety scores were used to determine the subjects’ general level of anxiety. The STAI scores range from a minimum of 20 points to a maximum of 80 points.

**Rating of the odour samples**

In the second part of the experiment, the frozen axillary pads containing the women’s odour samples from both films were put in odourless plastic bottles, which were then heated to 37°C (human body temperature). These bottles were presented to independent female raters. Three pads per donor were presented to 62 raters (aged 18–72 years, mean age: 22.02; S.D. = 7.51). The combination of bottles for rating (two horror samples, one neutral sample and vice versa) was chosen randomly. In a triple forced choice test the raters were asked to assess if there was a difference in the smell of the three bottles. They were told to write down their first impression of each odour, even if there was no consciously detectable smell. Then they had to rate each bottle by a) intensity of smell, b) pleasantness c) “smells like sex”, d) “smells like aggression” e) “smells like fear” on 10 cm long analogue scales [21]. Finally the raters had to fill in questionnaires concerning their smoking habits, age, cycle day, cycle length, diseases of the respiratory tract, etc.

**Results**

**Fear**

Analysis of State-Anxiety Scales (STAI) scores before and after the horror film, shows that [t] participants actually experienced fear: the mean state anxiety value before the horror film is significantly lower than after the movie (T-test for dependent variables: n=41; t=-5.18; df=40, p=0.000; Mean state value before horror film: 33.71; sd=7.54; mean state value after the film: 42.33; sd=10.98). Effect size is a raise in 14.37 % of the possible range. See figure 1.

![Figure 1: State-Anxiety values (SA) before and after the horror film (T-test for dependent variables: n=41; t=-5.18; df=40, p=0.000; Mean state value before horror film: 33.71; sd=7.54; mean state value after the film: 42.33; sd=10.98).](image-url)
In the control situation, the “neutral” film, no significant changes in the subjects’ anxiety scores before and after the presentation could be found (T-test for dependent variables: n=42; t=-0.176; df=41; n.s.; mean state value before film: 31.93; sd=5.81; mean state value after film: 32.10; sd=5.41).

When we compare the changes in the State-Anxiety scores before and after the film presentation across experimental conditions, we also find a significant difference. The changes in state anxiety scores in the horror situation are distinctly larger than in the control situation (T-test for dependent variables: n=41; t=-14.535; df=40; p=0.000; Mean difference horror: 8.73; sd=10.8; mean difference controls: 0.17; df=6.13).

Moreover, there are significant differences in the film assessment of the horror film and the neutral film. The test subjects rated the horror film as significantly less boring (Wilcoxon; n=41; Z=-5.579; p=0.000), significantly more frightening (Wilcoxon; n=41; Z=-5.579; p=0.000), significantly more suspenseful (Wilcoxon; n=41; Z=-2.398; p<0.02) than the neutral film. Thus we can conclude that the horror film actually induced fear in our subjects.

Cortisol

When we compare the subjects’ cortisol levels before and after each film presentation, we find that the cortisol concentrations decrease significantly in both the test (Wilcoxon; n=41; Z=-3.920; p=0.000; mean cortisol level before film: 4.6; sd=3.28; after film: 3.57; sd=2.54) and the control situation (Wilcoxon; n=42; Z=-5.370; p=0.000; mean level before film: 4.96; sd=3.09; mean level after film: 3.19; sd=2.06).

A correlation analysis between the changes in cortisol levels and the changes in state-anxiety scores during the horror film shows no significant results (Spearman: n=41; r=-0.176; p>0.05) and no significant correlations between state-anxiety scores and cortisol levels after the horror film could be found (Spearman: n=41; r=-0.21; p>0.05).

At this point it has to be mentioned that the well-known circadian rhythm in cortisol concentrations [22] was also present in the findings. A significant decrease in cortisol concentrations occurred with the daytime (see table 1). Closer examination of cortisol changes during movie presentation reveals that the mean decrease in cortisol differs significantly between the horror and the control situation. During the pre-

### Table 1: Correlations between daytime and cortisol levels (Spearman).

<table>
<thead>
<tr>
<th>Correlation</th>
<th>n</th>
<th>Mw</th>
<th>Sd</th>
<th>rs</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (ng/ml) at beginning of horror film and the beginning time of horror film</td>
<td>41</td>
<td>4.60</td>
<td>3.28</td>
<td>-745</td>
<td>000</td>
</tr>
<tr>
<td>Cortisol (ng/ml) at the end of horror film and end time of horror film</td>
<td>41</td>
<td>3.57</td>
<td>2.54</td>
<td>-595</td>
<td>000</td>
</tr>
<tr>
<td>Cortisol (ng/ml) at beginning of neutral film and beginning time of neutral film</td>
<td>42</td>
<td>4.96</td>
<td>3.09</td>
<td>-676</td>
<td>000</td>
</tr>
<tr>
<td>Cortisol (ng/ml) at end of neutral film and the end-time of neutral film</td>
<td>42</td>
<td>3.19</td>
<td>2.06</td>
<td>-652</td>
<td>000</td>
</tr>
</tbody>
</table>

### Table 2: Differences in the qualitative assessment of the odour samples (U-test).

<table>
<thead>
<tr>
<th>Film</th>
<th>n</th>
<th>Mw</th>
<th>Sd</th>
<th>MR</th>
<th>Z</th>
<th>p</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>horror</td>
<td>638</td>
<td>47.38</td>
<td>29.34</td>
<td>667.30</td>
<td>-2.948</td>
<td>&lt;.01</td>
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<tr>
<td>neutral</td>
<td>635</td>
<td>42.63</td>
<td>28.57</td>
<td>606.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pleasantness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>horror</td>
<td>635</td>
<td>35.96</td>
<td>21.80</td>
<td>613.52</td>
<td>-2.044</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>neutral</td>
<td>633</td>
<td>42.11</td>
<td>20.97</td>
<td>655.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aggression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>horror</td>
<td>633</td>
<td>23.01</td>
<td>26.20</td>
<td>653.58</td>
<td>-1.965</td>
<td>&lt;.05</td>
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<tr>
<td>neutral</td>
<td>633</td>
<td>20.72</td>
<td>25.43</td>
<td>613.42</td>
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<tr>
<td>sex</td>
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<tr>
<td>horror</td>
<td>635</td>
<td>21.06</td>
<td>24.73</td>
<td>636.35</td>
<td>-1.811</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>neutral</td>
<td>633</td>
<td>21.39</td>
<td>24.39</td>
<td>632.64</td>
<td></td>
<td></td>
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<td>fear</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>horror</td>
<td>634</td>
<td>25.61</td>
<td>27.02</td>
<td>649.58</td>
<td>-1.574</td>
<td>&gt;.05</td>
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<tr>
<td>neutral</td>
<td>632</td>
<td>23.76</td>
<td>26.39</td>
<td>617.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
sentation of the horror film, the subjects’ cortisol levels dropped significantly less than during the control film (Wilcoxon; n=41; Z=−2,138; p<0,05; mean difference horror film: 1,03; sd=1,84; mean difference control: 1.76; sd=1,58).

**Odour assessment experiment**

In order to find out if women were able to detect a difference between the “neutral” smell and the “frightened” smell of the pads, we used a triple forced choice test. This setting implies that a right decision by chance would happen with a probability of 33%. We found that the odour assessments were significantly more often correct than it could be expected by mere chance probability (One-Sample T-test: n=41; t=3,305; p=0,002; Mean=42,67; Sd=18,73).

Changes in participants’ cortisol levels during the acquisition of the odour samples had no influence on odour assessment, as no significant correlations could be found between changes in cortisol levels in both the horror and the neutral film situation and the percentage of correct decisions (Horror film: n=20; r=0,132; p>0,05; Neutral film: n=21, r=−0,148; p>0,05).

If we look at the qualitative rating of the odour samples, we find significant differences between the assessments of the “horror” and the “neutral” samples (see table 2). We find that the odour samples from the horror film presentation were rated as significantly more odourous (U-Test: n=635; Z=2,948; p<0,01; MR horror film: 667,3; MR neutral film = 606,56), and less pleasant (U-Test: n=633; Z=2,044; p<0,05; MR horror film= 613,52; MR neutral film= 655,54) than the samples taken after the presentation of the neutral video. Although there are no differences in the ratings “smells like fear” (U-Test: n = 632; Z=1,574; p>0,05; MR horror film= 649,58; MR neutral film = 617,37) and “smells like sex” (U-Test: n=633; Z=−0,181; p>0,05; MR horror film=636,35; MR neutral film=632,64) between the test situations, the women rated the samples from the horror film as more “aggressive”, than the neutral ones (U-test: n=633; Z=1,965; p<0,05; MR horror film= 653,58; MR neutral film = 613,42).

**Discussion**

The first question of interest in this study is if the test subjects were really frightened by the test situation. The results of the STAI show that the subjects experienced a significant increase in their state anxiety levels in the test situation. We can therefore conclude that the horror film was indeed suitable to evoke fear in the female subjects. This result is consistent with the result of Hubert & de Jong-Meyer [23] who found that “[...] unpleasant film stimuli reliably induce negatively valenced mood changes.”

Moreover, the fear-evoking effect of the horror video is reinforced by the results of the subjects’ film assessments, as they rated the horror movie as significantly less boring, more frightening, suspenseful and less funny than the neutral film.

Changes in cortisol levels have been used as an indicator for the experience of fear in many studies. In our experiment, saliva samples were drawn in an interval of about 70 minutes, just before and after the film presentation. Contrary to our expectations, the mean cortisol concentration exhibited a decline in both the test and the control situation. This result becomes clearer if we look at our significant negative correlation between cortisol levels and daytime, a well-known and often described connection (e.g. [22]). Hubert and de Jong-Meyer [6] found similar results: in their study, the subjects’ cortisol levels dropped continuously for forty minutes after the start of the experiment and then slowly raised again, but never again reached the starting point, not even after 180 minutes. The authors explain this result by the circadian rhythm of cortisol, with highest levels in the morning and lowest ones in the evening.

It is important to notice that in our experiment the decline in cortisol levels was significantly less during the presentation of the horror film than during the presentation of the neutral one. This result indicates that the HHN axis was indeed stimulated by the horror film and thus “worked against” the natural decline of cortisol with daytime. The search for a dedicated “fear pheromone” seems to be promising – the result that there are no correlations between the amount of experienced fear and cortisol, nor between the ability to detect fear and cortisol levels exclude a mere effect due to cortisol alone.

In the second part of the experiment, the odour assessments, we find that the female subjects were indeed able to differentiate between the odour samples from the horror film and the neutral film situation. The expected 33% of correct assessments by pure chance were significantly exceeded in our experiment. This finding can be interpreted as a strong hint that humans could use olfactory signals to inform their environment about their internal state, in our case, fear.

In addition to this effect, we find differences in the qualitative assessments of the odour samples between the test and the control situation. The women rated the smell of the pads from the horror situation as significantly stronger and more unpleasant than the pads from the neutral situation, and the odour reminded them of “aggression”. This can be taken as another hint that the odour we release in fearful situations is indeed special. Additionally, Panksepp [4] assumes a strong link between the “fear-” and the “rage-circuits” of the brain, which could also lead us to the assumption that the odour production that perhaps accompanies such emotions could have a similar link.

A summary of the results of this study suggests that humans are indeed able to smell their conspecifics’ fear, and can tell a difference between “fear” and
“non-fear” from body odours. It seems likely that this olfactory signal could be a “pheromone”, as it is hard to think of other plausible biological explanations for its production. But of course it has to be stated at this point, that our experiment cannot prove the fear-pheromone hypothesis, as a pheromone – per definition – has to evoke a reaction in the receiver, either a change in behaviour, or/and in physiology. This question could only be answered by follow-up studies.

What we know at the moment, as many studies in the last few years have pointed it out, is that the human sense of smell has by far been underestimated in the past and that humans – like other animals – use olfactory signals for the transmission of biologically relevant information. Our findings give yet another hint that olfactory communication in humans is of great importance.

REFERENCES
Effect of hydrocortisone on the activity of some lysosomal enzymes in mice

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Key words: hydrocortisone; lysosomes; cathepsin D and L; alanine aminopeptidase; leucine aminopeptidase; N-acetyl-β-glucosaminidase; lysosomal arylesterase and lysosomal lipase; homeostasis; mice

Abstract

OBJECTIVES: Changes in the activity of cathepsin D and L, alanine aminopeptidase, leucine aminopeptidase, N-acetyl-β-glucosaminidase, lysosomal arylesterase and lysosomal lipase in the liver and kidney of unselected and selected mice, subjected to 7.5 mg/kg b.w. of hydrocortisone injection for 4 and 8 days.

METHODS: The homogenates of the liver and kidney were subjected to differentiated centrifuging and determination of studied enzymes.

RESULTS: Injection of hydrocortisone caused an increase in the activity of all investigated lysosomal enzymes in the liver and kidney of mice.

CONCLUSION: The reactions of selected mice were stronger in comparison with unselected ones. The highest increase in the activity investigated enzymes was observed after 8 days of hydrocortisone injection.
Introduction

Glucocorticosteroids, and first of all hydrocortisone (cortisol), have influence on the metabolism of proteins, lipids and carbohydrates, and only in physiological concentrations plays an important role in regulatory processes, such as for instance in maintaining intracellular homeostasis [1–4]. The metabolic effects of hydrocortisone action arises from the type of the target tissue. For example, in muscles, adipose and lymphatic tissues it reveals catabolic activity, but in liver it stimulates synthesis and glycogen storage [5–7]. In physiological conditions adrenal cortex secretes about 20 mg of hydrocortisone within twenty-four hours, but during stressful reaction its secretion can increase even 10–times [8].

Environmentally unfavourable effects on animals cause, among much else, stress responses. One of the cell arrangements which take part in those responses is the lysosomal compartment. The physiological importance of the lysosomal structure has been described by numerous authors, which agree that it is the principal site of intracellular degradation processes [9–15]. It also a terminal compartment for the intracellular transportation of newly synthesised lysosomal enzymes [16].

In our studies we observed the activity of some lysosomal enzymes of mice subjected to hydrocortisone injections as model factors affecting homeostasis of an organism. The aim of our experiment was to determine the influence of the time of administration of exogenous hydrocortisone on the activity some proteolytical enzymes, glycosidases and lipases in the liver and kidney of mice selected on economic feed consumption and unselected ones, whose parents were matched at random.

Material and methods

The experiment was carried out on 30 56-day-old males mice from a line selected for 12 generations for economic feed consumption and 30 unselected mice, whose parents were chosen from a random match. The animals were bred in the Institute of Genetics and Animal Breeding, the Polish Academy in Kielce. They were constantly maintained in an artificial microclimate near Warsaw, Poland, with constant access to water. All animals received good veterinary care.

Mice were divided into groups (I–III selected mice, and IV–VI unselected mice; n = 10 in each group) and injected intraperitoneally daily (8:00 a.m.) according to the following scheme:

- I control 250 µl 0.9% NaCl
- II hydrocortisone 7.5 mg/kg b.w. 4 days
- III hydrocortisone 7.5 mg/kg b.w. 8 days
- IV control 250 µl 0.9% NaCl
- V hydrocortisone 7.5 mg/kg b.w. 4 days
- VI hydrocortisone 7.5 mg/kg b.w. 8 days

The mice of experimental groups (II, III, V, VI) received daily 7.5 mg/kg b.w. of exogenous hydrocortisone (Hydrocortisonum hemisuccinatum, Pharmaceutical Company Jelfa, SA, Poland).

The mice were killed by breaking the spinal cord, and the slices of the liver and kidney were perfused with 0.9% NaCl solution cooled to +5°C. The liver and kidney slices were suspended in 0.1 M phosphate buffer cooled to +5°C at pH 7.0 (500 mg tissue/5ml buffer), and homogenized in a Potter homogenizer with a teflon piston at 200 rot./min. The liver and kidney homogenates were subjected to differentiated centrifuging according to [17].

In the lysosomal fractions of liver and kidney the activity (nmol/mg of protein/hour) of cathepsin D and L (Cath. D, EC 3.4.23.5 and Cath. L, EC 3.4.22.15) according to [18]; alanine aminopeptidase (AAP, EC 3.4.11.2) according to [19]; leucine aminopeptidase (LAP, EC 3.4.11.1) according to [20]; N-acetyl-β-glucosaminidase (NAG, EC 3.2.1.30) according to [21]; lysosomal aryls-terase (EL, EC 3.1.1.2) and lysosomal lipase (LL, EC 3.1.1.3) according to [22].

Protein was also determined in the lysosomal fractions [23]. All substrates were from Serva Feinbiochemica GmbH & Co., Heidelberg, Germany. The results obtained were analyzed statistically according to Student’s t test.

The experiment was approved by the Ethics Commission for Animals Research of the Owiętokrzyska Academy in Kielce.

Results

As can be seen from Tables 1–4, hydrocortisone injection for 4 and 8 days caused statistically confirmed an increase in the activity of all the investigated lysosomal enzymes in liver and kidney of selected and unselected mice. The highest increase in the activity was observed in the liver and kidney of selected mice after 8 days of hydrocortisone injection.

Discussion

The mechanism of hydrocortisone action on the course of inflammatory, immune and neoplastic processes has not been explained yet; however, anti-inflammatory properties of this hormone deserve a special attention in relationship to its participation in the inhibition of the immune reactions of organism connected with the grafts rejection [24–27]. In physiological concentrations, hydrocortisone contributes to the maintenance of the homeostasis of the metabolism of carbohydrates, proteins and lipids. Many data show that it plays the part of a stabilizer of cellular membranes – especially lysosome ones [28]. In such a situation, usually active lysosomal enzymes remain imprisoned within the lysosomal membranes, the effect of which can be, among others, the inhibition of the exudative phase in the course of allergic reactions and acute and chronic inflammatory states [29–30].
Use of pharmacological doses of hydrocortisone, which exceed the physiological secretion of adrenal cortex may lead to disturbances of intracellular homeostasis and, in effect, to changes in the activity of the investigated lysosomal hydrolases [31]. A prolonged hydrocortisone administration in doses exceeding physiological ones leads to hyperglycaemia and adrenogenenic diabetes because it lowers the use of glucose by cells, its transport through cellular membranes, and contributes to the decrease of glycolysis in peripheral tissue [32–34]. Hydrocortisone may also cause essential changes in the metabolism of proteins, increasing their catabolism and mobilization of amino acids mostly in muscles and bone tissue, as well as intensifying transformations of amino acids in hepatocytes [35].

The results obtained show that the injection of hydrocortisone caused a significant increase in the activity of all the investigated lysosomal enzymes. Our investigation has noted an increase in the activity of both cathepsins (Cath. D and L), aminopeptidases (AAP and LAP), lysosomal lipases and also lysosomal glycosidase (NAG), hydrolizing glycoproteins and glycolipids [36]. Increase in the activities of lysosomal lipases is connected with the properties of hydrocortisone, which determines, among others, the course lipolysis and gluconeogenesis. The observed increase in the activity of both lysosomal lipases in the liver and kidney of mice subjected to the activity of this hormone is most likely connected with the mobilization of lipids and their intensive degradation. Administration of hydrocortisone caused an increase in the range and rate of the synthesis of lysosomal lipase and lysosomal arylesterase in the liver and kidney of selected mice [37–39].

The observed changes of the activity of the investigated lysosomal enzymes after hydrocortisone injection suggest that, lysosomal compartment reacts as one of the first cytoplasmic systems by activating the resistance mechanisms in situations constituting a threat to the maintenance of the existing homeostasis, they are also connected with the labilization of lysosomal membranes and the increase of their permeability and a release of proteases to the cytosol. In effect, this disturbs the functioning of the lysosomal system and leads to an irreversible destruction of cells [40–42].

Our investigation has shown that mice selected for economic feed consumption re-

### Table 1. The activity of lysosomal enzymes (x ±SD) in the liver of selected mice (in nmol/mg of protein/hour) after 4 and 8 days of hydrocortisone injection; control = 100%; n in each group = 10;

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control four days</th>
<th>Control eight days</th>
<th>Hydrocortisone 4 days</th>
<th>Hydrocortisone eight days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cath.D and L</td>
<td>0.076 ± 0.014</td>
<td>0.092 ± 0.017</td>
<td>121 ± 0.112</td>
<td>147 ± 0.208</td>
</tr>
<tr>
<td>AAP</td>
<td>0.590 ± 0.201</td>
<td>0.646 ± 0.165</td>
<td>109 ± 0.799</td>
<td>135 ± 0.235</td>
</tr>
<tr>
<td>LAP</td>
<td>1.25 ± 0.277</td>
<td>1.98 ± 0.770</td>
<td>158 ± 2.75</td>
<td>220 ± 0.864</td>
</tr>
<tr>
<td>NAG</td>
<td>1.14 ± 0.193</td>
<td>1.19 ± 0.215</td>
<td>104 ± 1.34</td>
<td>117 ± 0.396</td>
</tr>
<tr>
<td>EL</td>
<td>1.06 ± 0.215</td>
<td>1.57 ± 0.197</td>
<td>148 ± 1.93</td>
<td>182 ± 0.421</td>
</tr>
<tr>
<td>LL</td>
<td>1.49 ± 0.320</td>
<td>1.51 ± 0.088</td>
<td>101 ± 1.56</td>
<td>105 ± 0.109</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01; *** P < 0.001 – statistically confirmed differences;

### Table 2. The activity of lysosomal enzymes (x ±SD) in the liver of unselected mice (in nmol/mg of protein/hour) after 4 and 8 days of hydrocortisone injection; control = 100%; n in each group = 10;

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control four days</th>
<th>Control eight days</th>
<th>Hydrocortisone 4 days</th>
<th>Hydrocortisone eight days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cath.D and L</td>
<td>0.067 ± 0.023</td>
<td>0.075 ± 0.015</td>
<td>112 ± 0.091</td>
<td>136 ± 0.203</td>
</tr>
<tr>
<td>AAP</td>
<td>0.390 ± 0.056</td>
<td>0.395 ± 0.158</td>
<td>101 ± 0.437</td>
<td>112 ± 0.101</td>
</tr>
<tr>
<td>LAP</td>
<td>1.09 ± 0.457</td>
<td>1.19 ± 1.03</td>
<td>109 ± 1.24</td>
<td>114 ± 0.499</td>
</tr>
<tr>
<td>NAG</td>
<td>0.690 ± 0.098</td>
<td>0.713 ± 0.196</td>
<td>103 ± 0.987</td>
<td>143 ± 0.200</td>
</tr>
<tr>
<td>EL</td>
<td>0.910 ± 0.076</td>
<td>1.03 ± 0.896</td>
<td>113 ± 1.09</td>
<td>119 ± 0.872</td>
</tr>
<tr>
<td>LL</td>
<td>2.33 ± 1.03</td>
<td>2.71 ± 0.977</td>
<td>116 ± 2.96</td>
<td>127 ± 1.22</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01; *** P < 0.001 – statistically confirmed differences;

### Table 3. The activity of lysosomal enzymes (x ±SD) in the kidney of selected mice (in nmol/mg of protein/hour) after 4 and 8 days of hydrocortisone injection; control = 100%; n in each group = 10;

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control four days</th>
<th>Control eight days</th>
<th>Hydrocortisone 4 days</th>
<th>Hydrocortisone eight days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cath.D and L</td>
<td>0.222 ± 0.050</td>
<td>0.380 ± 0.110</td>
<td>171 ± 0.410</td>
<td>185 ± 0.150</td>
</tr>
<tr>
<td>AAP</td>
<td>2.66 ± 0.546</td>
<td>3.15 ± 1.13</td>
<td>118 ± 3.25</td>
<td>122 ± 0.998</td>
</tr>
<tr>
<td>LAP</td>
<td>2.97 ± 0.410</td>
<td>3.49 ± 0.978</td>
<td>117 ± 3.58</td>
<td>120 ± 0.876</td>
</tr>
<tr>
<td>NAG</td>
<td>0.648 ± 0.146</td>
<td>0.836 ± 0.099</td>
<td>129 ± 0.901</td>
<td>139 ± 0.130</td>
</tr>
<tr>
<td>EL</td>
<td>1.52 ± 0.453</td>
<td>1.99 ± 0.900</td>
<td>131 ± 2.15</td>
<td>141 ± 1.22</td>
</tr>
<tr>
<td>LL</td>
<td>0.208 ± 0.101</td>
<td>0.290 ± 0.034</td>
<td>139 ± 0.299</td>
<td>144 ± 0.055</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01; *** P < 0.001 – the differences statistically confirmed;

### Table 4. The activity of lysosomal enzymes (x ±SD) in the kidney of unselected mice (in nmol/mg of protein/hour) after 4 and 8 days of hydrocortisone injection; control = 100%; n in each group = 10;

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Control four days</th>
<th>Control eight days</th>
<th>Hydrocortisone 4 days</th>
<th>Hydrocortisone eight days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cath.D and L</td>
<td>0.810 ± 0.067</td>
<td>0.886 ± 0.103</td>
<td>109 ± 0.900</td>
<td>111 ± 0.897</td>
</tr>
<tr>
<td>AAP</td>
<td>1.77 ± 0.870</td>
<td>1.80 ± 0.874</td>
<td>102 ± 2.03</td>
<td>115 ± 1.02</td>
</tr>
<tr>
<td>LAP</td>
<td>1.98 ± 0.911</td>
<td>2.23 ± 1.02</td>
<td>113 ± 2.13</td>
<td>107 ± 1.00</td>
</tr>
<tr>
<td>NAG</td>
<td>0.950 ± 0.220</td>
<td>1.13 ± 0.794</td>
<td>119 ± 1.12</td>
<td>118 ± 0.996</td>
</tr>
<tr>
<td>EL</td>
<td>0.590 ± 0.090</td>
<td>0.688 ± 0.099</td>
<td>117 ± 0.743</td>
<td>126 ± 0.197</td>
</tr>
<tr>
<td>LL</td>
<td>1.15 ± 0.796</td>
<td>1.39 ± 0.076</td>
<td>121 ± 1.43</td>
<td>124 ± 0.764</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.01; *** P < 0.001 – the differences statistically confirmed;
papers on this topic found in the literature [43–46].

As until now the mechanisms which control the activity of lysosomal compartment have not been explained, the investigation performed induces one to widen the range of investigation using the changes of the activity of lysosomal enzymes to the observation of the course of adaptation reaction in humans and of animals.

The mechanisms regulating the activity of the lysosomal system and the secretion of its enzymes still evoke considerable interest in numerous biochemical laboratories as indicated by the increasing number of papers on this topic found in the literature [43–46].

REFERENCES

4 Bozena Witek, Ewa Ochwanowska, Alina Slewa & Adam Kolataj