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Renal Failure as the Cause of Death in *Tupaia belangeri* Exposed to Persistent Social Stress*

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Summary. 1. An adult male *Tupaia belangeri* was introduced to another male which was an experienced fighter. The latter immediately attacked the newcomer and subjugated him (Figs. 1 and 2). The two animals were subsequently separated, so that the subordinate animal could see the victor permanently, but could not be attacked by him. The fight and subjugation were repeated every 1–2 days, in order to maintain dominance relationships. Changes in physiological parameters were determined by sacrificing some of the tree-shrews at 2–16 days after the first subjugation, 80–100 minutes before the onset of daily activity.

2. After the first subjugation, subordinate animals lie still in a corner for more than 90% of the daily activity-phase and follow the movements of the victors with their heads. The tail-hair is continuously raised, indicating persistent activation of the sympathetic nervous system. Subordinate animals eat as long as controls.

3. After 2–16 days, the skin-temperature of the subordinates abruptly falls from more than 35°C to less than 30°C within 24 hours. Cramps and paralysis appear in the extremities, the tree-shrews fall into coma and die. Wounds can be excluded as the cause of death.

4. The following physiological changes take place in the subordinate animals:

a) The animals lose body weight in a linear fashion. The rate of weight-loss varies considerably between animals (Fig. 3). The more weight an animal loses daily, the more rapidly it dies ($r = 0.89$; Fig. 4). Death is not a result of inadequate energy-supply (resp. hypoglycaemia).

b) In subordinates, the glycogen content of the liver is significantly below that of controls ($p \approx 0.003$; Table 1); but even dying animals may exhibit normal glycogen values. The glucose concentration of the blood is the same under both conditions (Table 1).

c) The haemoglobin content of the blood decreases during stress exposure ($r = 0.75$; Fig. 5). The decrease is the more rapid, the more weight a subordinate animal loses daily ($r = 0.68$). The decrease in haemoglobin concentration is primarily due to haemolysis, but erythropoiesis is also restricted (Table 2).

d) The kidney weight decreases with continued stress; animals sacrificed more than 10 days after their first subjugation have kidneys approximately 17% lighter than those of controls ($p < 0.05$; Fig. 8).

e) During the course of stress, urea-N content of the blood increases (Fig. 6). The more rapidly the urea-N increases, the more rapid is the decrease in blood haemoglobin content ($r = 0.89$). Subordinate animals can move around normally with values up to 150 mg urea-N/100 ml blood, and they look healthy. If the concentration rises above this level, symptoms of imminent death appear, and the animals die.

* Dedicated to Professor Dr. H. Autrum on the occasion of his 65th birthday.

f) The uraemia results from renal insufficiency which can be traced histologically to a decrease in renal blood flow. The pathological histology of the kidneys is described (Figs. 9–16). The extent of histologically demonstrable damage to the kidneys shows a correlation with the increase of blood urea-N.

5. In control animals with surgical impairment of renal function, the urea-N content of the blood increases by about 140 mg/100 ml per day (Fig. 7). Death occurs after 36–48 hours, as soon as the urea-N level has risen above 180 mg/100 ml. The symptoms of death corresponded to those seen in subordinate tree-shrews. This leads to the following two conclusions: With social stress, death is actually a result of renal failure. The urea-N content of subordinate animals can rise nearly as rapidly as in operated tree-shrews. Thus, social stress can lead to a more-or-less complete renal failure.

6. *Discussion:* Death in tree-shrews under persistent social stress is brought about by uraemia which results from a decrease in renal blood flow. This is interpreted, to some extent at least, as a consequence of renal vasoconstriction due to high activity of the sympathetic nervous system (through renal nerves as well as through adrenal catecholamines). Moreover, indications from literature are presented, suggesting that in natural populations of various animal species it is again renal function which is disrupted at times of high population density or with pronounced social stress. The evidence from natural populations, when examined along with the findings from tree-shrews, show the great significance which social stress may have in the origin of renal disease—possibly in man as well as in animals.

Zusammenfassung. 1. Ein adultes männliches *Tupaia belangeri* wurde zu einem fremden, kampferprobten Männchen gesetzt. Dieses griff den Neuling augenblicklich an und unterwarf ihn (Abb. 1 und 2). Anschließend wurden beide Tiere durch eine Zwischenwand getrennt, so daß der Unterlegene den Sieger zwar sehen, nicht aber von ihm attackiert werden konnte. Der Kampf mit Unterwerfung wurde alle 1—2 Tage wiederholt. Änderungen physiologischer Parameter wurden bestimmt; hierzu wurde ein Teil der Tupajas 2—16 Tage nach ihrer ersten Unterwerfung 80—100 min vor ihrem Aktivitätsbeginn getötet.

2. Nach der ersten Unterwerfung liegen unterlegene Tupajas mehr als 90% der Aktivitätsperiode still in einer Ecke und verfolgen die Bewegungen des Siegers mit dem Kopf. Die Haare auf dem Schwanz sind andauernd aufgerichtet; dies deutet auf eine ständige Aktivierung ihres sympathischen Nervensystems hin. Unterlegene Tiere fressen ebenso lange wie Kontrollen.

3. Nach 2—16 Tagen sinkt die Hauttemperatur der Unterlegenen in etwa 24 Std von mehr als 35°C auf weniger als 30°C ab, es zeigen sich Lähmungen und Krämpfe in den Extremitäten, die Tiere fallen in Koma und sterben. Verwundungen sind als Todesursache auszuschließen.

4. Folgende physiologischen Veränderungen wurden bei den Unterlegenen festgestellt:

a) Das Körpergewicht nimmt im Verlauf der Belastung gleichmäßig ab; das Ausmaß der Gewichtsabnahme variiert von Tier zu Tier beträchtlich (Abb. 3). Je mehr ein Tier täglich Gewicht verliert, desto eher stirbt es ($r = 0{,}89$; Abb. 4). Der Tod ist jedoch nicht die Folge einer unzureichenden Energieversorgung (bzw. Hypoglykämie).

b) Der Glykogengehalt in der Leber liegt bei Unterlegenen signifikant ($p < 0{,}003$) unter dem Wert der Kontrollen (Tabelle 1), jedoch können selbst sterbende Tiere noch „normale" Glykogenwerte haben. Die Blutzuckerkonzentration ist unter Kontroll- und Stressbedingungen gleich (Tabelle 1).

c) Der Hämoglobingehalt im Blut nimmt im Verlauf der Belastung ab ($r = 0{,}75$; Abb. 5). Der Abfall ist um so schneller, je mehr ein Unterlegener täglich an Gewicht verliert ($r = 0{,}68$). Der Abfall der Hämoglobinkonzentration beruht vor allem auf einer starken Hämolyse, jedoch ist auch die Erythropoese beeinträchtigt (Tabelle 2).

d) Das Gewicht der Nieren nimmt im Verlauf der Belastung ab; nach mehr als 10 Tagen liegt es signifikant um etwa 17% unter dem Kontrollwert ($p < 0{,}05$; Abb. 8).

e) Der Harnstoff-Stickstoffgehalt im Blut steigt unter Stress an. Je schneller dieser Anstieg ist, desto schneller nimmt der Hämoglobingehalt im Blut ab ($r = 0.89$). Bis zu Werten von 150 mg Harnstoff-N/100 ml Blut können sich die Tiere normal bewegen und machen einen gesunden Eindruck. Steigt die Konzentration darüber, so treten die Exitussymptome auf und die Tiere sterben.

f) Die Urämie beruht auf einer Niereninsuffizienz, die sich histologisch auf eine — von Tier zu Tier unterschiedliche — Drosselung der Nierendurchblutung zurückführen läßt. Die pathologischen Nierenveränderungen werden beschrieben (Abb. 9—16). Das Ausmaß der histologisch nachweisbaren Schäden zeigt eine Beziehung zum Anstieg des Harnstoff-Stickstoffes im Blut.

5. Der Harnstoff-N im Blut von Kontrollen, deren Nieren operativ ausgeschaltet wurden, steigt in 24 Std um etwa 140 mg/100 ml an (Abb. 7). Der Tod tritt nach 36—48 Std ein, sobald der Harnstoff auf mehr als 180 mg/100 ml angestiegen ist. Die Exitussymptome entsprechen denen unterlegener Tupajas. Hieraus ergeben sich zwei Folgerungen: Der Tod bei sozialem Stress erfolgt tatsächlich auf Grund einer Niereninsuffizienz. Der Harnstoff-N von unterlegenen Tieren kann fast ebenso schnell wie bei operierten Tieren ansteigen; die soziale Belastung kann daher offensichtlich zu einem nahezu vollständigen Nierenversagen führen.

6. *Diskussion:* Der Tod von Tupajas bei sozialem Stress wird durch eine Urämie bewirkt, die auf einer Verringerung der Nierendurchblutung beruht. Diese ist — zumindest zum Teil — durch eine Konstriktion der Nierengefäße erklärbar, die auf einer erhöhten Aktivität des sympathischen Nervensystems beruht. Es werden weiterhin Hinweise aus der Literatur dafür aufgeführt, daß auch in natürlichen Populationen verschiedener Tierarten die Funktion der Nieren in Zeiten hoher Bevölkerungsdichte bzw. bei starker sozialer Belastung beeinträchtigt ist. Dies deutet im Zusammenhang mit unseren Befunden an Tupajas auf die große Bedeutung hin, die soziale Belastungen — möglicherweise auch beim Menschen — für die Entstehung von Nierenerkrankungen haben können.

A. Introduction

Social contact in mammals can be a source of stress, to which the organism may adapt with hormonal changes. These adaptive responses can be interpreted on the basis of the stress concept developed by Selye (for summary, see Selye, 1950): They are primarily characterised by increased activity of the hypophyseal-adrenal system and a corresponding reduction in activity of the hypophyseal-gonadal system, depending upon the degree of stressor action.

Christian (1950) was the first to point out the great importance which such hormonal adaptations may have for self-regulation of populations: Increasing population densities, according to his concept, lead to an

increase in "social stress" and thus to a reduction in fertility and vitality, which operates against population increase (for summaries, see: Wynne Edwards, 1962; Christian, 1963; Barnett, 1964; Welch, 1964; Calhoun, 1971). Hormonal adaptation to social stress and associated changes in growth and fertility have been demonstrated in tree-shrews (*Tupia belangeri*) (Autrum and v. Holst, 1968; v. Holst, 1969).

If stress exceeds a certain level, adaptation of the organism is not feasible—at least over an extended period of time—and the individual will die. Stress of lethal intensity can also be produced through social interactions in mammals. Although, particularly in captivity, death can be brought about by persistent attacks and resulting wounds which the subordinate animal cannot escape, in most cases conflicts between conspecifics lead to dominance relationships and thus to extensive reduction of actual fighting. Yet, subordinate animals will still frequently die within a short time, even though they suffer no serious wounds (Barnett, 1958; Christian, 1963; Andrzejewski *et al.*, 1963; Reimer and Petras, 1967), and it has been demonstrated that this also happens under natural conditions (Calhoun, 1948). In tree-shrews, fighting between two animals (males or females) always results in extremely rapid subjugation of one by the other.

In a fairly large cage (floor more than 5 square meters), the victor will pay virtually no attention to the submissive animal, the latter, however, will cower in a corner which it leaves only to feed, and death will invariably ensue within a few days (v. Holst, 1969).

The following paper is concerned with the cause of death of subordinate tree-shrews under such conditions. Since wounds and infections can be excluded, it must be assumed that it is the stress-induced physiological response itself which becomes destructive.

The results suggest that in *Tupaia belangeri* it is rapidly-appearing renal failure which results in the deaths of submissive animals.

B. Materials and Methods

1. Animal Maintenance

The experiments were carried out with male *Tupaia belangeri*, most of which were captured as adults in Thailand. Some animals were bred in captivity in the Institute colony. Before the investigations were started, all tree-shrews were maintained in isolation for more than three months, in order to exclude as far as possible the after-effects of capture, transport and acclimatization to the laboratory environment. All animals were weighed once a week before the onset of their daily activity period. The investigations were carried out with animals whose body-weight had remained constant (variation less than $\pm 3\%$) for more than 6 weeks prior to the experiments, and whose weights lay in the range 180–220 g. All experimental animals were more than 9 months old.

The tree-shrews were kept singly in cages with a floor area 50×100 cm^2 and height of 50 cm. The front wall and the floor of each cage were made of cage-wire,

permitting the passage of faeces and food remains, and the remaining walls consisted of wooden panels. The animals were screened from direct physical contact with one another, and they were also visually isolated. To the front wall of each cage a small wooden box was attached, in which the animals slept at night or hid after disturbance during the day. This sleeping-box could be removed from outside; this made it possible to obtain the animals without any delay due to capture.

Day-length was maintained constant, with artificial light, at $L:D = 12:12$ hours. Room temperature was kept at $23 \pm 3°$ C.

Food and water were always available ad libitum, unless otherwise stated. The food consisted of chopped beef heart, diced fruit of various kinds, and mineral and vitamin additives (see v. Holst, 1969).

2. Experimental Procedure ("Psychosocial Stress")

The experiment was conducted in a room which was visually and acoustically isolated from the animal maintenance room. It contained one cage (floor area: 100×100 cm^2; height: 60 cm), which could be divided by a vertical cage-wire partition into two compartments, each of which corresponded approximately in size to the standard maintenance cages. This experimental cage was continuously occupied by a male ("fighter") which had already proved to be successful in fighting. A strange male was then introduced, was usually attacked at once and subjugated in less than 2–3 minutes (for details see p. 241). The two animals were then separated from one another by the vertical partition, so that the submissive animal could see the victor continuously, but was protected from physical attack. Every 1–2 days, the fight and subsequent submission sequence was repeated between the two animals.

Some of the experimental animals were left in this situation until they died; but most animals were removed from their sleeping-boxes 2–16 days after onset of stress exposure and sacrificed in the manner described below. Animals living in the maintenance room served as controls.

3. Chemical Determinations

Both experimental and control animals were killed 80–100 minutes prior to onset of the light phase (= activity phase), by incision of the left carotid artery. Blood was then collected for the various tests and blood-extraction always completed less than 2 minutes after removal of the sleeping animal from the nest-box. The liver was removed and deep-frozen in an alcohol/carbon dioxide mixture, less than 30 secs after completion of blood-collection. Various organs were dissected out, roughly cleaned of fat and attached connective tissue, weighed and then fixed in Bouin's solution. After embedding in Paraffin, the organs were sectioned (sections 3–6 μ) and usually stained with Haematoxylin-Eosin[1].

With some animals, small blood samples were taken from the tail arteries several times during the course of the investigations. This was also done without exception 80–100 minutes before the onset of the light-phase.

The following parameters were measured:

Glycogen content of the liver, using Pfleiderer's enzymatic technique (1962).

Glucose concentration of the blood, using the hexokinase (HK) method developed by the Boehringer company (Mannheim).

Urea-nitrogen content of the blood, measured semi-quantitatively with Azostix-strip-test (Merck Company, Darmstadt and Ames Company, Elkhart) and with

1 I wish to thank Mrs. Wallisch for preparation of the histological material.

Boehringer-test-combination (Boehringer Company, Mannheim). The Azostix-test only requires a drop of whole blood and little more than one minute is required for the determination of urea-N. According to Dugle and Free (1969) this method will yield values comparable to those obtained with conventional methods. During the course of our experiments we found that the values obtained with the strip-test were about a third to a quarter the urea-nitrogen levels as determined by Boehringer-test-combination (correlation coefficient $r=0.96$; $p<0.001$; $n=46$), in tree-shrews as well as in rats and mice. An increase in concentration, however, is readily visible with the Azostix-method. Changes of urea-nitrogen levels during persistent stress were followed with the strip-test alone, since the quantity of blood required for measurement by other methods could not be obtained from a single animal on several occasions during the course of the experiment. These values were not corrected by any factor, but marked distinctly as Azostix-values (see Fig. 6).

Haemoglobin-content of the blood, estimated as haemiglobincyanide using the Asid company photometric method.

Number of leucocytes in the blood, counted in a Fuchs-Rosenthal counting chamber following staining with Türk's solution.

Number of erythrocytes in the blood, counted in a Thoma counting chamber following staining with Hayem's solution.

Number of erythroblasts in the blood, computed from their percentage relationship to the leucocytes and the absolute count of the latter per mm^3 of blood. The percentage representation of the erythroblasts was determined by blood-smears which were stained with Pappenheim's panoptic method (peroxydase-reaction, modification II, following Undritz's "Hämatologische Tafeln Sandoz" for eosinophil cells).

C. Results

1. Behaviour

If an adult male tree-shrew is placed in the cage with the fighter the newcomer is attacked at once (Fig. 1). This leads to a bout of biting, which usually ends after a few seconds with submission of the introduced animal. From this time onwards, the subordinate animal utters "fear squeals" (Sprankel, 1961) whenever approached by the dominant, and attempts to flee—unsuccessfully under these experimental conditions. While attempting to escape, the subordinate animal is repeatedly bitten on rump, thighs and the root of the tail; but such biting produces only superficial abrasions. After some time (30–120 sec), the escape attempts cease and the submitting animal lies motionless—and often silent—on the floor. However, the prostrate animal is quite capable of movement, since it will always leap up and flee if the victor is removed. The motionless posture does not prevent the dominant animal from subsequently launching rapid series of attacks on the submitting animal (Fig. 2); but even in such attacks, the latter sustains only superficial wounds, and no bleeding occurs. Nevertheless, there is a rapid appearance of irregular breathing, respiratory arrest and death. In 6 experiments, this occurred 45–200 sec after the first attack made by the fighter. The accompanying symptoms resemble those of "vagus death" observed in

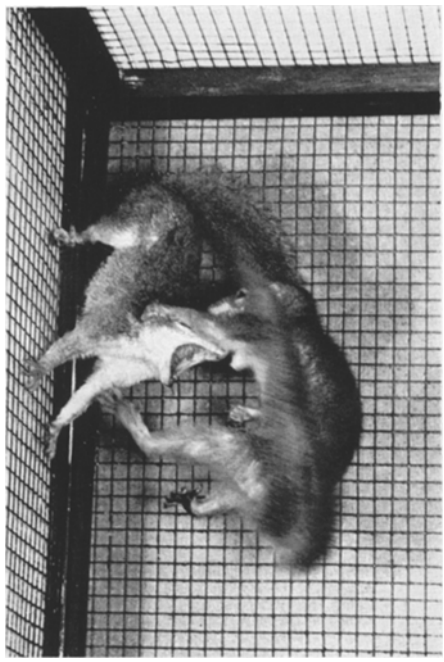

Fig. 1

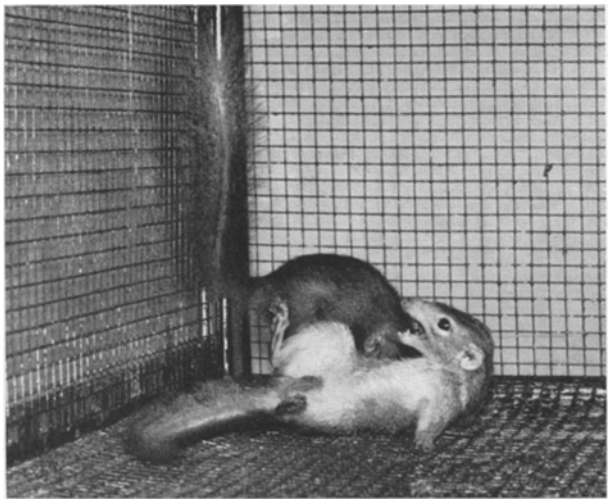

Fig. 2

Figs. 1 and 2. Fighting between two male tree-shrews. Attack by the "fighter" (Fig. 1) and submission of the newcomer (Fig. 2)

Norway rats under quite different stress conditions by Richter (1957), and which Barnett (1958) has already discussed in connection with death in Norway rats following social conflict.

If the two animals are separated by the cage-wire partition (see methods) after the first complete submission, the subordinate animal always recovers within 5–10 minutes. However, the subordinate's behaviour changes markedly: It hardly moves at all, spending more than 90% of the daily activity-phase lying motionless in a corner and following the movements of the victor with its head. Under control conditions, resting time represents only about 20–30% of the daily activity-phase. Approach of the victor on the other side of the partition sometimes leads to "fear squealing", but otherwise the subordinate animal gives the general impression of apathy. The hairs on the tail and the rest of the body are raised continuously, giving the subordinate animal an untidy appearance, which is further emphasised by the fact that submissive animals virtually cease to groom themselves (0–300 sec per 12-hour activity-phase, compared with 1000–3000 secs in controls). Water-intake appears to increase; but quantitative measurements have yet to be carried out. A subordinate animal eats—at least during the first few days—just as long as control animals (1500–2500 sec per day), but the food is eaten far more rapidly. Marking with the sternal and abdominal glands and with urine, which is normally observed at high frequency, is completely lacking in subordinate animals from the time of submission onwards.

In the experiments, the subordinate animal was usually exposed to the fighter once a day by removing the cage-wire partition, and this always led to instantaneous attack by the dominant animal. The submissive animal still occasionally attempted to escape from these renewed attacks, but it normally accepted them with complete passivity. Since animals exposed to renewed attacks die following very few attacks, after exhibiting the symptoms described above (respiratory arrest, etc.), the attack time was kept very short (10–30 sec). Such attacks were merely intended to maintain the dominance relationship. Nevertheless, during the course of the investigations, three animals died within 10–15 sec during these renewed attacks. For this reason, very weak tree-shrews and those which were highly aroused (pronounced hair-raising; fear squealing, etc.) were not exposed to further attacks.

The changes in weight and physiological state of the subordinate tree-shrews which will be reported subsequently are mainly brought about by the continuous threatening impact of the victor and not merely by the summed after-effects of daily defeats:

In three cases, subordinate animals obviously learned that a victor separated by a cage-wire partition is "harmless". Although they exhibited considerable losses in body weight during the period immediately

after subjugation and showed behaviour similar to that of the other submissive animals, they subsequently (after 3, 7 and 8 days respectively) ceased to respond with pilo-erection to the presence of the dominant, moved around normally in their cage-section and increased in body weight, despite the fact that they were exposed to particularly vigorous submissions every day. Thus, their responses corresponded to those of animals which are exposed to fighting daily, but which are separated for the rest of the time from the victor by an opaque partition (see Raab, 1971). Under such conditions, animals only exhibit mild weight-loss and very little change in behaviour.

The three tree-shrews were then placed in a large cage with a male which similarly subjugated them, but subsequently left them virtually undisturbed. All three animals died after a few days in this situation, in which there was a persistent possibility of attack by the dominant animal.

Excitation produced by the presence of the victor, if maintained continuously for several days, leads to pronounced weight-loss (see below) and to death within a period of 20 days: Of 17 animals, 3 died after 2—3 days, 10 died after 4—7 days and 4 died after 10—16 days. 15 of these animals died at night or shortly before the onset of activity and 2 died during the day.

1—2 days before death, a reduction in skin temperature is already present, a drop to 30.0° C from 35.4° C (= average normal temperature) occurring. At the same time, the movements of the subordinate animals become faltering and locomotion difficult. Running, climbing or jumping are impossible, although at the beginning threat will provoke an attempt. Roughly 20 hours before death, convulsions and paralysis appear in the extremities. In most cases, the animals will no longer react to their environment, allowing themselves to be handled, by this stage being in deep coma.

Death itself was not observed as most animals died during the night. In addition, the animals were usually sacrificed when death appeared to be imminent, in order to obtain data on physiological changes.

2. Body Weight

The body weight of subordinate animals decreases regularly from the time of submission to death, although there is virtually no locomotion. The daily weight-loss is more-or-less constant for any individual animal throughout the entire experiment; but there are considerable differences between individuals (examples: Fig. 3). Daily weight-loss can be between 1.6 and 6.5% of the initial body weight. In animals which died under these conditions, the mean value was a loss of 3.5% of the initial body weight per day.

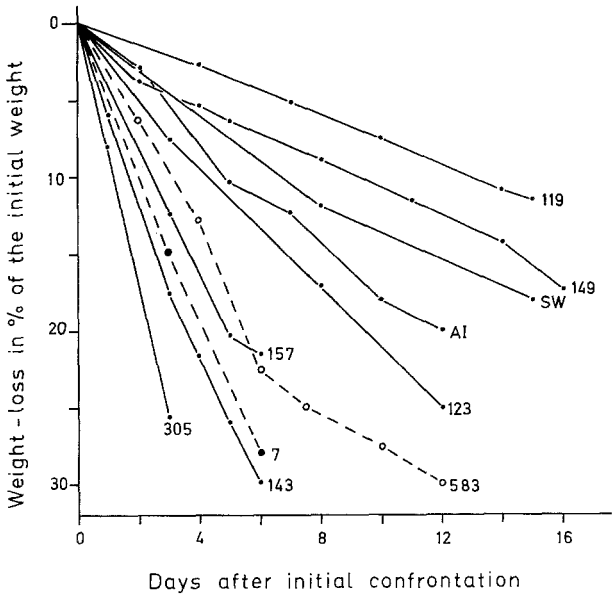

Fig. 3. Loss of body-weight in male tree-shrews with various treatments: • under persistent social stress; food and water ad libitum, ○ under control conditions without food; water ad libitum, ● under control conditions without food and water

There is an extremely good correlation between number of days between initial confrontation and death and daily weight-loss (correlation coefficient $r = 0.89$; $p < 0.001$; $n = 17$): The more weight an animal loses per day, the sooner it dies (Fig. 4).

From this, it could be concluded that there is metabolic collapse due to insufficient energy-supply. The animals do, in fact, continue to take food up to the day of death (faeces were almost always present in the colon of dying or sacrificed animals); but digestion and resorption might be disrupted to such an extent by stress that the food is no longer processed. For example, Baker (1952) found with rats under stress that there was a pronounced restriction of nutritional processing. However, insufficient energy-supply seems to be unlikely as a cause of death in tree-shrews. Even when no food is available at all, animals of comparable initial body weight live longer than 12 days under control conditions (4 experimental animals). During the course of time, they lose more than 25% of their body weight and attain a "minimal weight" of approximately 130 g before dying (see Fig. 3).

Under continuous stress, fat and muscle tissue are resorbed in just the same way as is the case with hunger. After 4–7 days, there is usually no fat left in the body, and the resorption of the skeletal musculature

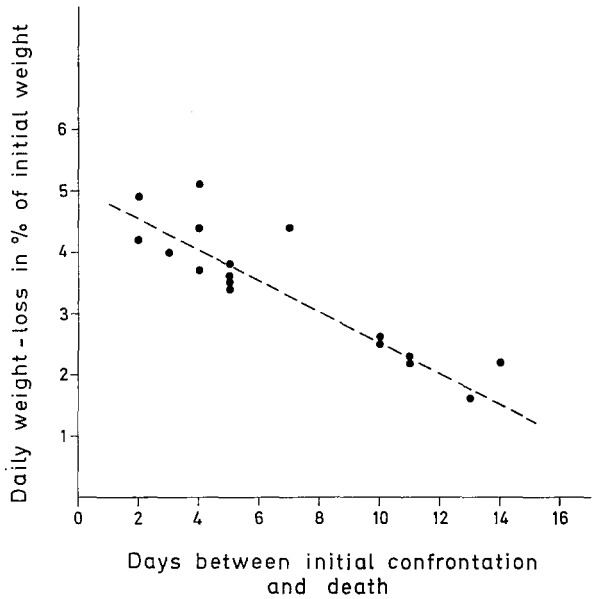

Fig. 4. Time of death of subordinate tree-shrews under persistent social stress, related to the daily weight-loss of individual animals

leads to a conspicuous alteration of bodily appearance (see v. Holst, 1969). However, the animals usually die before reaching their "minimal weight"; that is, before the available energy reserves have been completely exhausted. With those animals which die within 6 days—i.e. those which have the highest daily weight-loss—fat tissue is frequently still present in the abdominal cavity at death.

It is also conceivable that mobilisation of these energy reserves is hindered by a lack of adrenal hormones. For example, Christian (summary: 1963) concludes that the release of glucocorticoids and/or catecholamines can be impaired to such an extent by persistent social stress that it results in deficient gluconeogenesis, with resulting hypoglycaemia. However, in tree-shrews the concentration of glucocorticoids in the serum—following a temporary reduction in the first days of stress exposure—is ultimately greatly increased (v. Holst, 1972). For this reason alone, insufficient mobilisation of glycogen reserves is improbable. But the strongest evidence against inadequate or non-existent energy-mobilisation is provided by the blood sugar concentration (see below).

3. Liver Glycogen and Blood Sugar

The glycogen-content of the liver varies over a wide range in tree-shrews under control and stress conditions (Table 1). In 80% of animals

Table 1. Liver glycogen and blood sugar concentrations in male tree-shrews under control conditions and under persistent social stress

Experimental situation	Number of animals	Mean value	Standard error of the mean	Range
	Glycogen in mg per g of liver			
Control	12	56.5	6.8	14.5–87.7
Social stress	23	28.8	4.9	1.1–68.3
	Glucose in mg per 100 ml blood			
Control	10	100.5	2.1	90–110
Social stress	16	91.4	4.1	52–110

under stress, the value is lower than the mean control level, and the mean values of the two groups of animals are significantly different ($p = 0.003$)[2]. Death was not caused by exhaustion of glycogen reserves in the livers: Glycogen values of less than 3 mg/g were found after 2 days in some animals as well as after 7—14 days in others. However, even dying animals (skin temperature below 30° C; convulsions and coma) still had on occasion more than 35 mg of glycogen per g of liver.

Under stress conditions, the blood sugar content of tree-shrews is almost as high as that of control animals (Table 1). The mean value for the stressed animals is, in fact, somewhat lower; but this difference is entirely due to the low values for 3 of the 16 experimental animals, whose blood contained between 52 and 73 mg glucose per 100 ml. These low values were not a result of absence of glycogen reserves in the livers of such animals, since they still contained 10.8–56.0 mg/g of glycogen.

By contrast, even those animals with extremely low glycogen values (3 animals with less than 2 mg/g) still had normal blood sugar values of 90—105 mg/100 ml.

In 7 cases, blood was taken for glucose estimation several times during the course of stress exposure (every 2–4 days). These values also showed a generally constant and normal blood sugar concentration throughout the experiment.

In no case did animals with paralysis of the extremities, muscular convulsions and cold skin surface (i.e. with typical symptoms of hypoglycaemia) exhibit low blood sugar values.

Although these findings do not exclude the possibility that the blood sugar levels of individual animals might drop just before death, hypoglycaemic shock cannot be the cause of death in tree-shrews.

2 Statistical evaluation of differences was carried out by the *t*-test throughout.

4. Haemoglobin Content of the Blood

Control animals possess 19.4 ± 0.2 mg haemoglobin per 100 ml blood ($n = 20$). During the course of stress exposure, the haemoglobin content decreases (correlation coefficient $r = 0.75$; $p < 0.001$; $n = 46$).

After two days, the haemoglobin content is already significantly ($p < 0.001$) below the initial value, having reached 14.2 ± 1.3 g/100 ml ($n = 5$), and—despite considerable variation—it is still above the mean value measured when animals were sacrificed after 10 days of stress exposure ($p < 0.05$) (see Fig. 5).

The extent of haemoglobin reduction varies considerably from animal to animal. The more body weight an animal loses each day, the more rapid is the reduction in haemoglobin content of the blood (correlation coefficient $r = 0.68$; $p < 0.001$; $n = 31$). Since there is a very good correlation between the extent of daily weight-loss and the time of death (p. 244), mortality could be interpreted as a product of reduced or arrested oxygen-supply to the tissues. The experimental animals are, as previously mentioned, cold to the touch more than 20 hours prior to death, and at this time they have haemoglobin values of less than 10 g/100 ml blood. Although such anaemia certainly weakens the organism, the fall in skin-temperature, convulsions and eventual death in tree-shrews cannot be attributed to the decrease in haemoglobin concentration. For example, the blood of 4 animals just prior to death (skin temperature below 30° C, convulsions and coma) showed a haemoglobin content only marginally below the normal value (15.8–18.1 g/100 ml). On the other hand, in 3 animals with haemoglobin values of 5.0–9.5 g/100 ml normal skin temperature (34.4–35.3° C) was still present and they exhibited no behavioural signs of impending death.

Reduction in haemoglobin content is primarily due to a corresponding decrease in the number of erythrocytes. A slight alteration in haemoglobin concentration of the individual erythrocytes cannot yet be excluded, however.

The reduction of the number of erythrocytes—and thus the fall in haemoglobin concentration—cannot be attributed to loss of blood.

In no case did confrontation lead to wounds producing externally recognisable bleeding, and no macroscopic signs of internal bleeding were evident. In addition, even the extraction of large quantities of blood during the course of several days in control animals did not produce such low haemoglobin values as those evoked by social stress: When collecting blood by sectioning the carotid artery, 8–10 ml of blood were obtained. The total blood volume in a tree-shrew is about twice this amount. Even collection of approx. 2 ml of blood daily only lowers the haemoglobin value of control animals to about 12 g/100 ml after 5 days, whereas the haemoglobin value of tree-shrews under stress fell to 5.5–15.8 g/100 ml after this period of time.

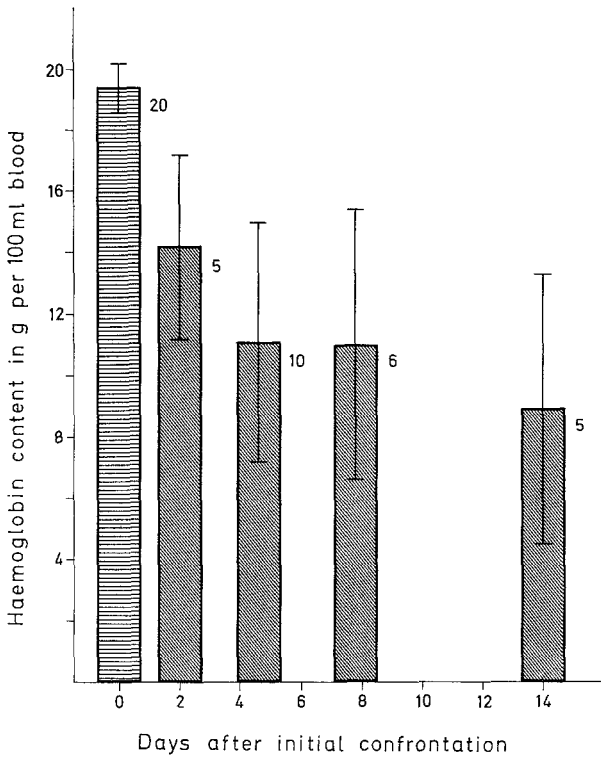

Fig. 5. Haemoglobin concentration of the blood of male tree-shrews related to the duration of social stress. Each bar shows the mean value for various categories, with the standard deviations and the number of experimental animals. Categories: controls; animals at 2, 3–6, 7–10 and 11–16 days after onset of stress exposure

Reduction in haemoglobin concentration under stress is obviously the result of some degree of haemolysis, which often results in a yellowish discoloration (icterus) of the blood serum. Provisional results indicate that there is a relationship between the intensity of haemoglobin loss and increase in bilirubin concentration in the blood. However, bilirubin levels have not yet been quantitatively estimated.

All animals in which the haemoglobin content had dropped to 5–10 g/100 ml in less than 5 days had an intensely yellow serum. Repeated blood extraction on successive days showed that the yellowish discoloration of the serum disappears when the haemoglobin concentration has dropped to such low values ($n = 5$). In animals with a slower rate of reduction of haemoglobin—still showing more than 12 g/100 ml after 5 days—no yellowish discoloration of the serum appeared.

A serum which has a pronounced yellowish colour always produces discoloration of the bodily organs. In 9 of 23 experimental animals,

Table 2. Number of erythroblasts and haemoglobin concentration in the blood of male tree-shrews under various conditions (see text for details)

	Number of animals	Number of erythroblasts per mm³ of blood			Haemoglobin content in g per 100 ml blood	
		Mean value	Standard error of the mean	Range	Mean value	Range
Controls	17	140	27	32–550	19.4	16.8–21.0
Stressed animals	21	86	17	0–306	13.1	9.1–17.0
"Blood-donors"	2	965	—	730–1200	11.7	10.0–13.4
"Recuperators"	4	340	161	120–820	13.8	10.2–18.1

there was a marked greenish-grey discoloration of the kidneys and—to a lesser extent—of the liver. In 2 cases, the liver had an intense yellow-green appearance.

The gall-bladder was always extremely large, and the normally invisible gall-bladder capillaries were in many cases recognisable macroscopically owing to their expanded contents. The yellowish discoloration of the serum is thus probably the result of "cumulative jaundice" produced by extensive haemolysis.

Under persistent stress conditions erythropoiesis is reduced, or not activated to the extent which would be expected from the low haemoglobin concentration. The differences between control and stressed animals are not significant, however—perhaps simply because of the small number of measurements available (Table 2).

Whereas each animal possesses at least 30 erythroblast per mm³ of blood under control conditions, with more than 30% of the subordinate animals it was impossible to find any erythroblast at all. This reduction in erythroblast count is particularly surprising because a reduction in erythrocyte count normally leads to a very marked increase in the number of erythroblasts in the blood.

We found, for example, that with 2 animals whose haemoglobin concentration had been reduced by 30 and 45% respectively through extraction of large quantities of blood (approx. 8 ml in 4 days) there was an increase in the erythroblast count to 730 and 1200 per mm³ of blood, respectively ("blood-donors", Table 2).

In the same way, the haemoglobin concentration of tree-shrews which have low values when removed from stress conditions will begin to rise after only 2 days. Correspondingly, the number of erythroblasts in the blood is markedly increased compared to the stressed animals ("recuperators", Table 2).

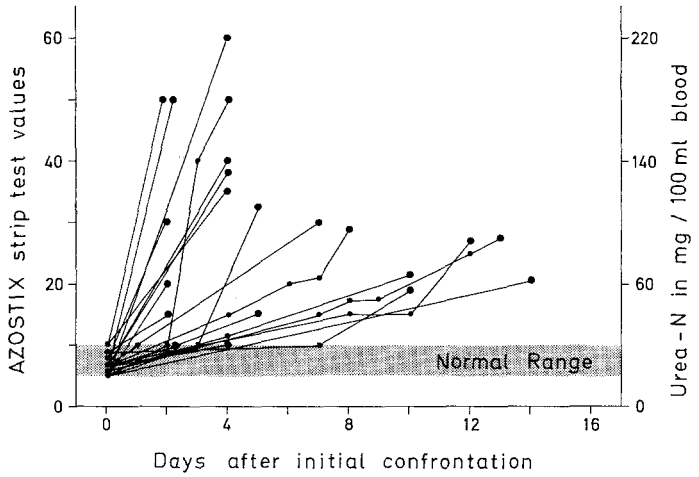

Fig. 6. Urea-nitrogen content in the blood of subordinate tree-shrews during social stress. The values were determined by the Azostix-strip-test. This method is relatively inexact (see methods). The urea-nitrogen content may vary within a range of $\pm 15\%$

Thus, the low haemoglobin values are also partially a result of reduced or arrested erythropoiesis.

5. Function and Morphology of the Kidney

a) Urea-Nitrogen Content of the Blood

Control animals showed 5–20 mg urea-N in 100 ml of blood (morning value: 13.2 ± 0.8 mg/100 ml; $n = 21$). During the day the urea-N can rise (e.g. after feeding) up to 40 mg/100 ml (day values: 25.0 ± 2.2 mg/100 ml; $n = 12$).

Under conditions of persistent social stress, the level of blood urea-N increases; but there is a considerable variation between animals in the extent of this increase (Fig. 6): whereas the blood of some subordinate animals possesses more than 150 mg urea-N in 100 ml of blood after 2 days, others only attain a value of 60–80 mg/100 ml after 10–14 days.

Fighting alone does not lead to an increase in urea-nitrogen content: Thus, the victors never exhibited levels of urea-N higher than 35 mg/100 ml. Similarly, there was no increase in three animals which had each been exposed on 5–8 successive days to serious subjugation, but were unable to see the victor in the intervening periods.

While subordinate animals can move around normally with urea-N values of less than 150 mg/100 ml (Azostix-value: approx. 40), values

above this are accompanied by symptoms of impending death (convulsions, paralysis, coma), and death follows within a few hours.

An increase of breakdown products of protein metabolism in the blood is referred to as uraemia. It is characterized by poisoning of the organism, which is expressed through tiredness, muscular spasms in the extremities and comatose condition. Death always follows after a deep coma—often as a result of cardiac arrest (Zollinger, 1966; Doerr, 1970).

These symptoms of uraemia correspond so closely with our findings on tree-shrews that it can be concluded: *Under social stress, tree-shrews die from uraemia.*

It is not yet clear whether it is the accumulation of urea and creatinine per se that causes the death or rather the accumulation of other toxic substances (such as organic acids or phenols). However, the concentration of blood urea-N is a suitable index of the severity of uraemia independent of whether the symptoms are caused by urea and/or other toxic substances or by corresponding changes in electrolyte- and water balance.

Such uraemia can have different causes:

Catabolic Uraemia. There is a weak correlation between weight-loss and increase of the urea-N content of the blood (correlation coefficient $r = 0.51$; $p < 0.01$; $n = 25$): The more rapidly an animal loses weight, the faster the increase in urea-N in the blood. This relationship could be explained on the basis of catabolic uraemia, since enhanced catabolism leads to an increase of protein breakdown products in the blood.

In order to test this possibility, 5 animals were kept for up to 10 days without food, and 2 others were fed only with a protein-rich diet. The urea-nitrogen content of the blood was measured at regular intervals. Although all animals lost 3.0–3.5% of their initial weight per day and resorbed their skeletal musculature to a great extent during the process, the urea-N did not rise above 45 (25,6–43,1) mg/100 ml in any instance. Thus, the great increase in urea-N during stress cannot be explained on the basis of increased formation of protein breakdown products.

Exsiccosis. Inadequate or the absence of water results in water retention by the kidneys (due to the effect of vasopressin). This can lead to reduced excretion of breakdown products of protein metabolism.

As observations showed, tree-shrews do drink water under persistent stress, but the uptake could be insufficient. To determine the increase in urea-N in animals under permanent thirst, we kept 4 controls without food and water. This resulted in a loss of 6–7% of body weight per day. The levels of blood urea-N rose only slightly in all animals: after 2 days: 43.7 ± 1.8 mg/100 ml; after 4 days: 49.1 ± 4.7 mg/100 ml. The experiment was then terminated with 2 of the animals, because it showed that the increase of blood urea under stress could not be caused by

inadequate water uptake. Two animals were kept in the experimental situation until death occured after 6 and 7 days respectively.

Renal Insufficiency. Particularly during the first days after the onset of social stress, urine-production seems to be considerably reduced or even completely arrested. Animals which were sacrificed 2–6 days after the initial confrontation (approx. 2 hours before onset of activity) usually had a virtually empty bladder, whilst control animals always had a completely full bladder at this time. Quantitative investigation of urine-production during stress is in progress.

We obtained enough urine from 7 subordinate animals when they were sacrificed to conduct analysis. The urine of one animal was blood-coloured. Although the urine of the others was colourless, sedimentation of the urine showed a large number of erythrocytes in 4 animals and a few erythrocytes in two others. No erythrocytes were found in the urine of control animals ($n = 8$).

There is a very close negative relationship between urea-N and haemoglobin concentration in the blood: the faster the increase in concentration of urea-N, the more rapid the decrease in haemoglobin content of the blood (correlation coefficient $r = 0.89$; $p < 0.001$; $n = 22$).

This close correlation, along with restriction of urine-production indicates impaired renal function: Kidney diseases almost always lead to anaemia, which is primarily a result of some degree of haemolysis. Damage to the renal tubules also affects erythropoiesis, by reducing the formation and release of erythropoietin (e.g. see: Zollinger, 1966; Erslev, 1970; Penington and Kincaid-Smith, 1971; Takaku *et al.*, 1971).

Liver Insufficiency. Even with complete intact kidneys, the urea-N content can increase in case of severe liver damage. In many cases, there is combined insufficiency of the liver and kidneys ("hepatorenal syndrome"). However, our findings with tree-shrews can be explained—at least for the first few days of stress exposure—entirely by renal damage, on the basis of microscopic evidence (see p. 255) and the data presented above. But this does not exclude some participation by the liver.

b) Surgical Impairment of Renal Function

In order to obtain some indication of the degree to which renal function is disrupted by social stress, 4 control animals were examined for increase in blood urea-N after the renal arteries, veins and the ureters on both sides had been ligatured.

All animals became active again less than 1 hour after the operation (narcosis with ether), moving around normally in the cage and eating well. After 30–40 hours, their movements became faltering, convulsions and paralysis appeared, and soon afterwards the animals fell into coma.

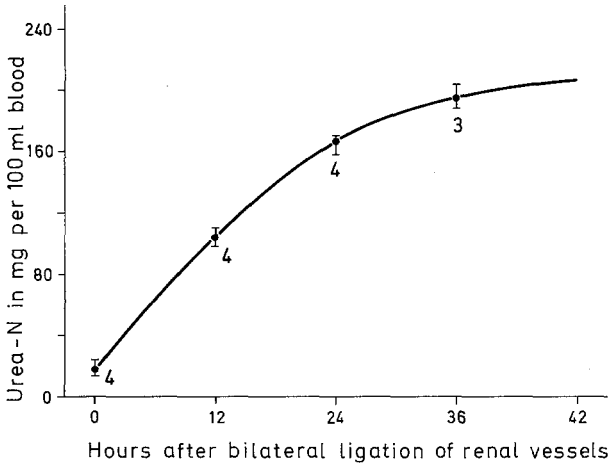

Fig. 7. Increase in blood urea-nitrogen in male tree-shrews following bilateral ligation of renal vessels and urethers. (Range and number of experimental animals is indicated.)

Despite the maintenance of glucose values of more than 50 mg/100 ml blood, the skin temperature rapidly declined to less than 30° C. The haemoglobin content of the blood decreased by about 50% in 24 hours (falling on average from 14.5 to 7.5 mg/100 ml from the 12th to the 36th hour after operation). Internal haemorrhage may, however, be involved in this reduction. Death followed 36–48 hours after the operation.

The urea-nitrogen content of the blood increased more or less linearly after operation, rising by about 140 mg/100 ml in 24 hours (Fig. 7). At death, the urea-N concentration of the blood was always more than 180 mg/100 ml.

The behaviour of the animals, the fall in skin temperature and the urea-N values at death largely correspond to the conditions prevailing under persistent social stress. This provides support for the assumption that death in tree-shrews due to social stress should be interpreted on the basis of renal insufficiency.

The operated animals lost about 4.1 (3.2–4.8) % of their body weight in 24 hours. This degree of weight-loss lies in the upper range of daily weight-loss measured under conditions of social stress (see p. 244). Thus, urea-production (through protein-supply and endogenous protein catabolism) should be approximately the same in operated animals and in tree-shrews exposed to social stress.

The urea-N concentration of operated animals increases by about 140 mg/100 ml in 24 hours (Fig. 7). In extreme cases, nearly the same

rapid increase can be found with subordinate tree-shrews (see p. 251), which indicates that almost complete renal failure may be brought about by exposure to social stress. Correspondingly, a less rapid increase in urea-N under conditions of persistent stress indicates a lesser degree of renal insufficiency.

c) Weight and Appearance of the Kidneys

The weight of the kidneys of control animals varies between 1225 and 2021 mg; the mean weight is 1584 ± 72 mg ($n = 15$). In the course of stress exposure, the weight of the kidneys decreases (correlation coefficient $r = 0.35$; $p < 0.05$; $n = 48$). The kidneys of animals which were killed after more than 10 days of stress exposure were significantly ($p < 0.05$) lighter than those of control animals (by approximately 17%; Fig. 8), despite the great variation in weight.

The external coloration of the kidneys in subordinate tree-shrews is almost always lighter (pale brownish-green) than in those of controls. This suggests that there is reduced blood-supply to the renal cortex in submissive animals.

In 4 out of 9 animals which had survived for more than 10 days under social stress conditions, the renal cortex was fragmented by deep fissures, and the fragments were only loosely held together by the renal medulla (see Fig. 11).

Such "fragmented" kidneys were found in 2 animals which had already died 2–6 hours before dissection and in 2 animals which had been sacrificed while in coma. Even in the first two animals the condition of the kidneys was unlikely to have been due to post-mortem changes, since kidneys which were not removed from control animals until 24 hours after death never showed such changes.

d) Microscopic Condition of the Kidneys

The glomerular capillaries of subordinate tree-shrews are markedly distended in comparison with control animals, and contain very few erythrocytes or none at all (Figs. 13–15). In the Bowman's capsule there is usually accumulated material, indicating increased permeability of the basement membranes. In many cases, individual glomeruli are ruptured and collapsed, and erythrocytes can be seen in the tubules. As exposure to stress continues, the form of the glomeruli becomes increasingly irregular (Figs. 15 and 16) and glomerular capillaries sporadically disintegrate.

The lumina of the renal tubules are more-or-less distended in all animals exposed to persistent stress, whilst in controls, with a few exceptions, they have a closed appearance (Figs. 9 and 10).

The lumina of kidneys fixed after death do not correspond to the conditions in the living organism. In the latter, renal tubules are expanded. After arrest of

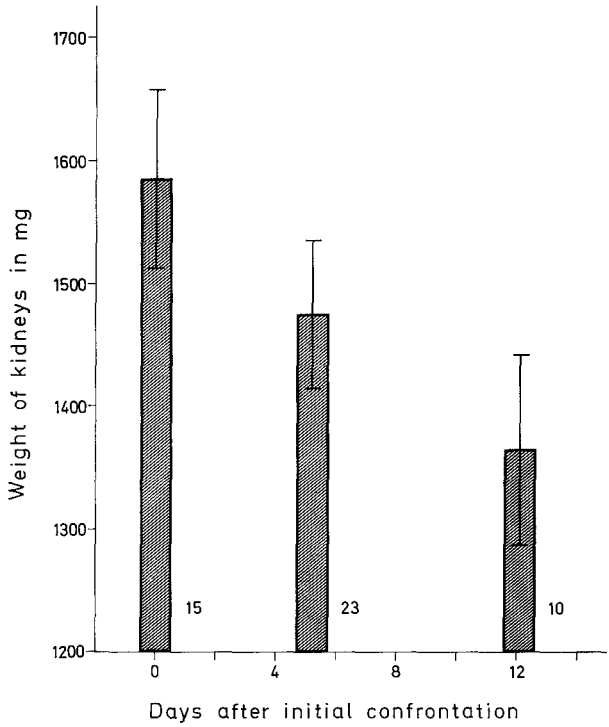

Fig. 8. Kidney weight in male tree-shrews during exposure to social stress. Each category shows the mean value with its standard error and the number of experimental animals. Categories: Controls; animals after 2–7 and 8–16 days

filtration, respectively of circulation following death, intact tubules resorb the filtrate which they contain and collapse, thus histologically exhibiting a narrow lumen. Damage to the tubules inhibits this supravital tubule collapse (Walther, 1965).

Particularly during the first 6 days, the tubules and the collecting ducts of the renal medulla are frequently completely packed with material, which is probably largely composed of disintegration debris of erythrocytes (e.g. Fig. 10). The form of the tubules becomes increasingly irregular during the course of stress (Figs. 15 and 16), and the tubule epithelium becomes reduced in thickness until it ultimately consists of no more than a thin layer.

After about 10 days of stress, the first signs of tubular necrosis can be recognized, and in subsequent days necrosis can become widespread (e.g. Fig. 12). However, apparently functional tubules can often be seen alongside completely necrotic areas.

Severe necrosis was in two cases an obvious cause of the "fragmentation" of the kidneys described above, since the disrupted cells formed large lacunae extending out to the surface of the kidney. In two other cases, however, the kidneys were fragmented without any signs of necrosis in the area concerned (e.g. Fig. 11).

There is a relationship between histologically demonstrable kidney damage and increase in urea-N content of the blood: The kidneys of controls contain almost exclusively nephrons with a functional appearance. With subordinates exhibiting an urea-N increase of less than 30 mg/100 ml in 24 hours, glomeruli of normal appearance may lie directly alongside functionally impaired glomeruli. Correspondingly, the tubules associated with the glomeruli have an intact appearance or exhibit distended lumina. The more rapidly the urea-N in the blood of a subordinate animal increases, the more glomeruli are ischaemic and the more advanced is ischaemia in individual glomeruli. (The quantitative relationships between morphology and function are now being studied in greater detail.) In extreme cases, all glomeruli are ischaemic or anaemic, and all renal tubules are expanded and thus non-functional (e.g. see Fig. 10); the urea-N content of such animals had always increased by more than 80 mg/100 ml in 24 hours. Thus the histological appearance of the nephrons and the increase in breakdown products of protein metabolism in the blood both indicate more-or-less complete renal failure.

D. Discussion

1. Psychosocial Processes as the Cause of Social Stress

The deaths of subordinate tree-shrews are brought about by the continued presence of the victor, since it has been shown that even 1–2 daily subjugations do not in themselves lead to death (p. 251). Therefore, in the experimental situation, stress is not due to active social interactions, but to perception and some central nervous process(es) based on experience and learning. This is particularly obvious from the following two observations:

Tree-shrews only die when they are exposed to the presence of the animal which has subjugated them. The continued presence of a strange "fighter" does not lead to the death of a subordinate animal even when the latter is subjugated once a day by the dominant animal with which it is actually acquainted.

If an animal learns that the dominant tree-shrew can no longer attack after separation by the cage-wire partition, the subordinate does not die but recovers again, despite severe subjugation encounters every day (see p. 243).

Bronson and Eleftheriou (1965) were the first to demonstrate that the sight of a dominant mouse can produce stress in another mouse,

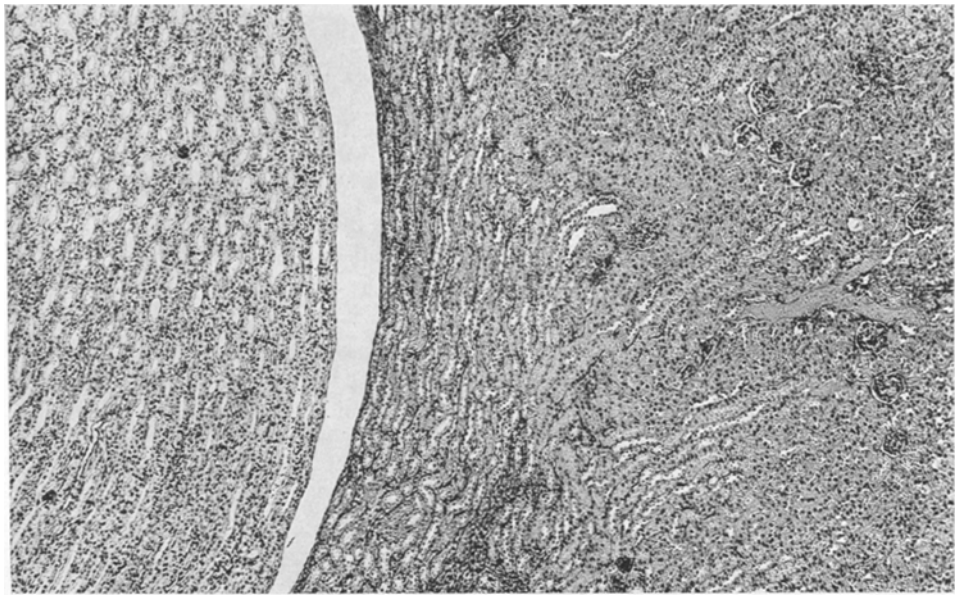

Fig. 9

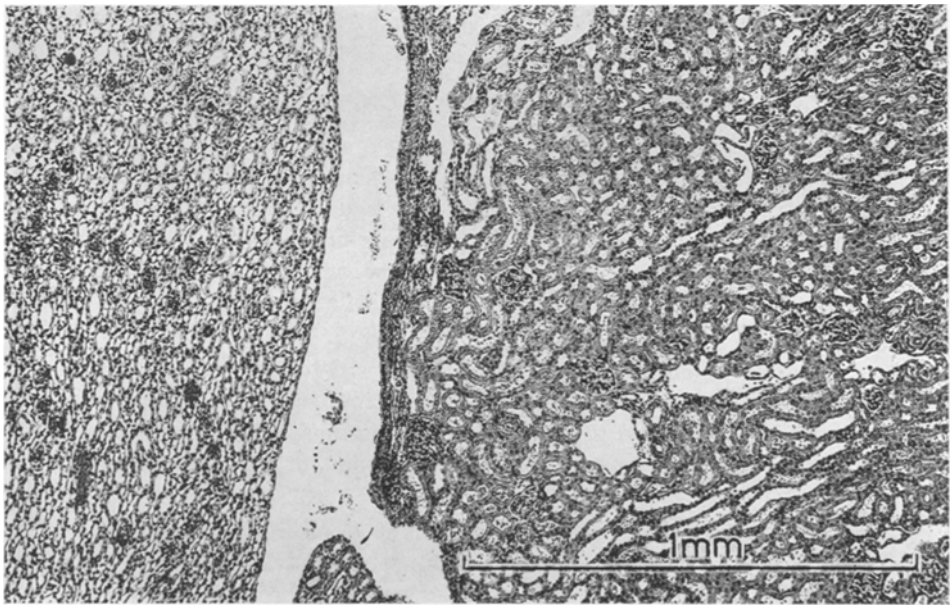

Fig. 10

Figs. 9 and 10. Sections through kidneys of male tree-shrews under control and stress conditions. Sections approx. 5 μ thick; staining with Haematoxylin/Eosin. Fig. 9. Control. Fig. 10. After two days of stress exposure. The tubules are distended. Cylinders can be seen in the renal medulla

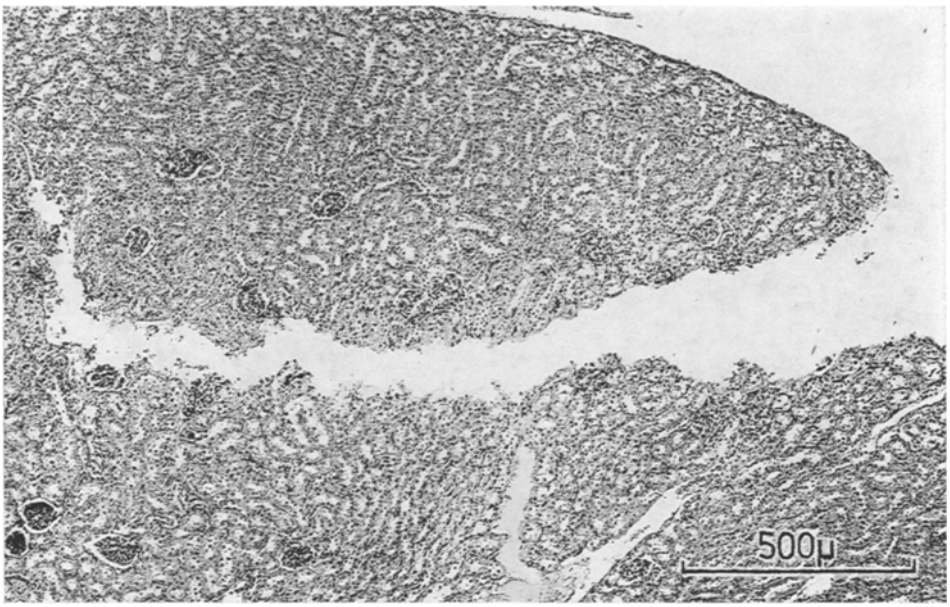

Fig. 11

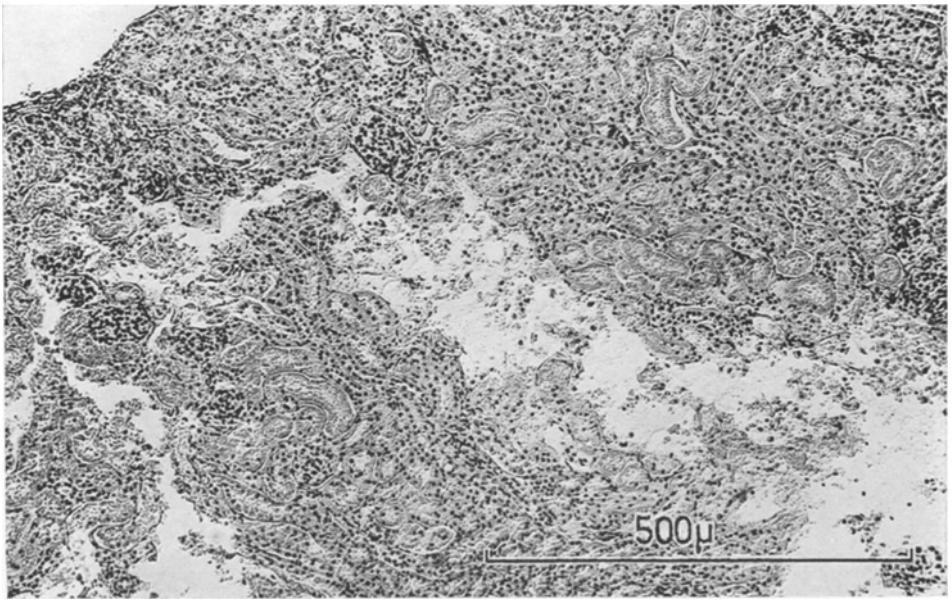

Fig. 12

Figs. 11 and 12. Sections through "fragmented" kidneys of subordinate tree-shrews. Sections 5 µ thick; staining with Haematoxylin/Eosin. Fig. 11. Kidney without necrosis after 14 days of stress exposure. Fig. 12. Kidney with severe necrosis after 11 days of stress exposure

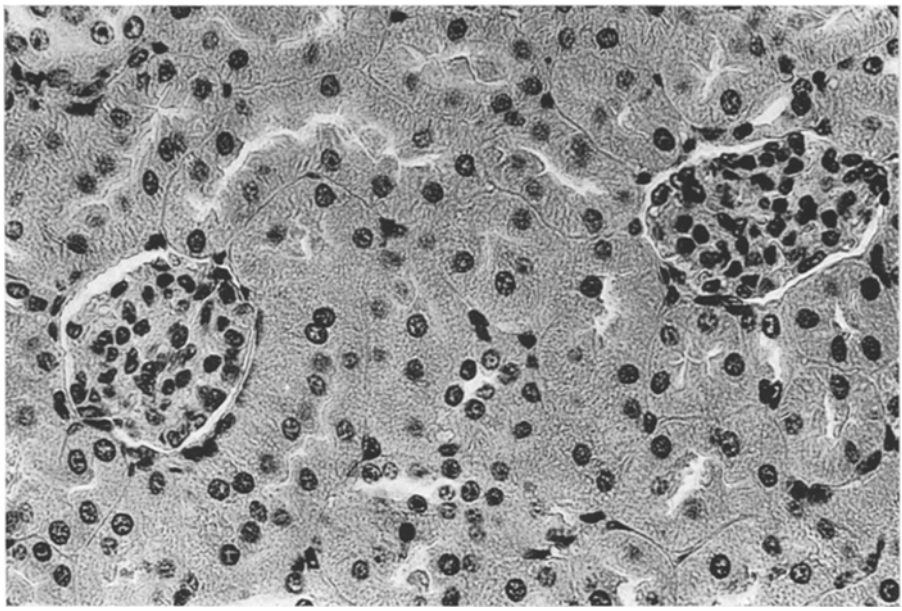

Fig. 13

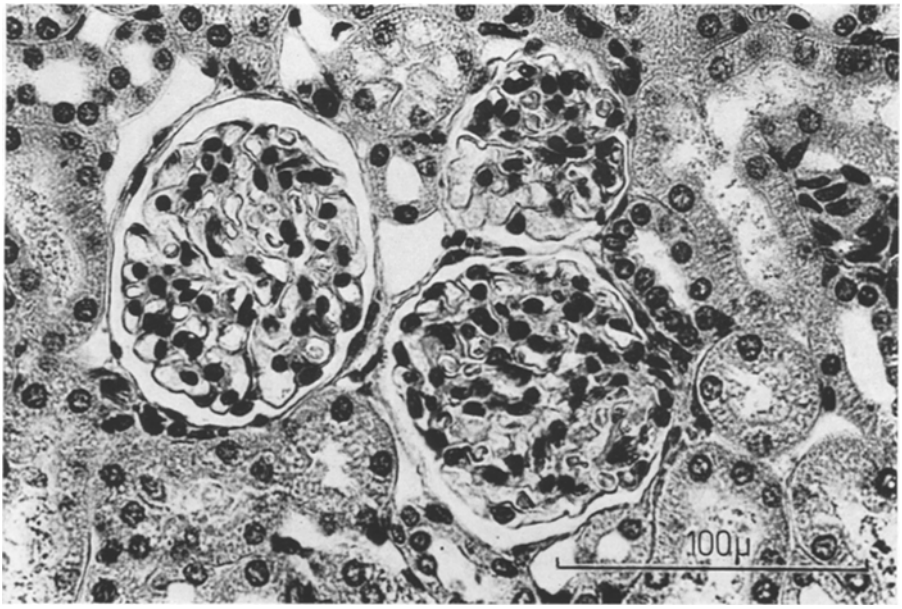

Fig. 14

Figs. 13–16. Nephrons of male tree-shrews under control conditions and after different periods of exposure to social stress. Sections approx. 3 µ thick; staining with Haematoxylin/Eosin; all sections same magnification. Fig. 13. Control animal. Fig. 14. After 2 days of stress. Fig. 15. After 4 days of stress. Fig. 16. After 12 days of stress

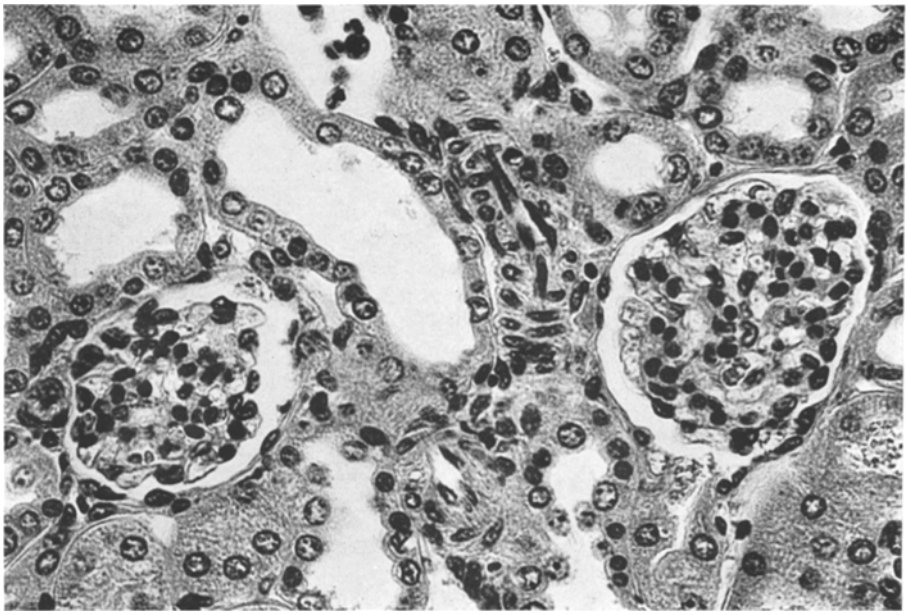

Fig. 15

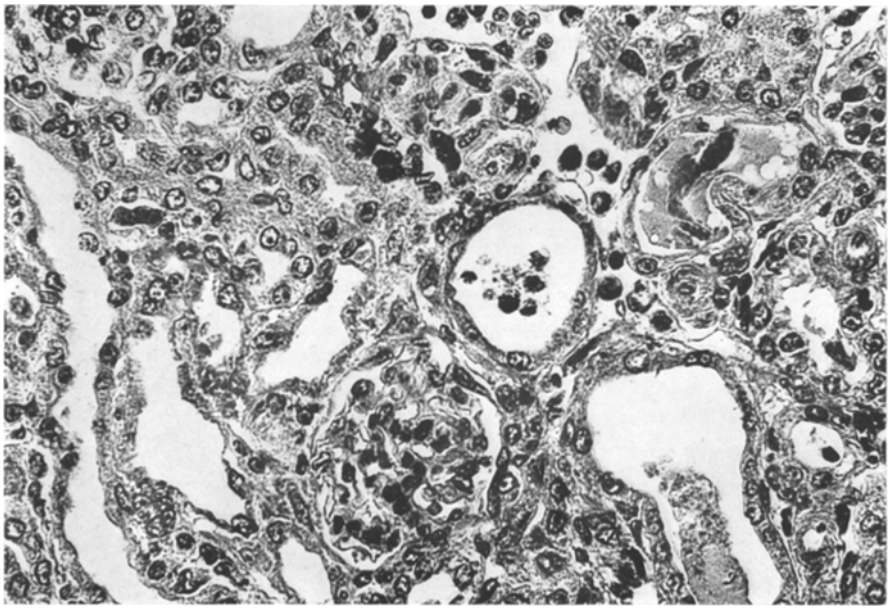

Fig. 16

which had previously been subjugated by the former: The hypophyseal-adrenal system of the subordinate animal is activated almost to the same extent as in actual fighting.

In tree-shrews, any activation of the sympathetic nervous system is accompanied by pilo-erection in the tail (v. Holst, 1969), and the subordinate animal actually exhibits this reaction continuously in the presence of the dominant.

In a situation of less intense stress (e.g. daily subjugation without continued presence of the victor) the tail-hair is not spread during the entire day, and physiological changes take place, the degree of which relates to the daily duration of activation of the sympathetic nervous system. In such cases the organism can adapt itself (see v. Holst, 1969 for details). Only the continuous activation over several days, as present in the above described experimental situation, leads to death. Thus, it is not the *intensity*, but the permanent *duration* of social stress which causes death in tree-shrews.

2. Physiological Cause of Death

a) Carbohydrate Metabolism

An increased level of activity in the sympathetic nervous system (indicated by the pronounced spreading of the tail-hair) is doubtless accompanied by release of adrenaline and noradrenaline from the adrenal medulla (summarised by Levi, 1967; Mason, 1968). Glucocorticoid hormones from the adrenal cortex are similarly discharged into the blood in increased quantities (v. Holst, 1972). Increased synthesis and release of these substances leads to a considerable increase in adrenal weight.

After only 2 days of stress exposure, the adrenal weight is significantly ($p < 0.001$) above the initial value of 18.0 ± 0.5 mg ($n = 18$). After 4 days, it attains a level (31.7 ± 0.9 mg; $n = 21$) which is maintained virtually constant until death.

Whilst the catecholamines stimulate glycogenesis in liver and skeletal muscle, glucocorticoides induce gluconeogenesis. This leads to a reduction in body weight which, in the present experiment, varies from animal to animal (see p. 244). Although there is a close correlation between the intensity of weight-loss and the time from the onset of stress to death (see p. 244), this does not mean that animals die from the exhaustion of mobilisable carbohydrate reserves (i.e. hypoglycaemia): even dying tree-shrews may have blood-sugar values which correspond to those of control animals. Nevertheless, in subordinate animals, the utilization of glucose is obviously so pronounced that it cannot be offset by the increase in fresh glucose production. This leads to a reduction in glycogen concentration in the liver (see p. 246).

A corresponding reduction in liver glycogen is also known from other animal species exposed to massive stress. For example, snowshoe hares

from collapsing populations always die within a few days after capture, with accompanying convulsions and paralysis, whilst this is not the case with animals taken from normal populations (Green and Larson, 1937; 1938; Green *et al.*, 1939). In the view of the authors cited, the considerably reduced glycogen content of the dying animals indicates "shock disease" (hypoglycaemic shock) as the cause of death in natural populations. With field-voles, too, individuals taken from collapsing populations exhibit reduced liver glycogen content and, in some cases, lowering of the blood sugar level. Frank (1953) also concluded from this that hypoglycaemic shock is the cause of population crashes, and this opinion is shared by Christian (1963). On the basis of his extensive investigations with rodents, Christian regards this "failure" of carbohydrate metabolism as the results of adrenal exhaustion.

This assumption is, however, based exclusively on morphological and histochemical investigations. In fact, there is often a lack of correlation between morphological and functional parameters of the adrenals (Elton *et al.*, 1959; Vecsei and Csalay, 1965; v. Holst, 1972). Exclusively morphological studies therefore do not justify such a conclusion.

But these indications of hypoglycaemia as the cause of death are, in themselves, inconclusive, since the glycogen content of the liver and the blood sugar concentration of snowshoe hares and voles often lie in the range of control animals. In addition, high insulin discharge in voles may lead to a reduction of liver glycogen and blood sugar, but this cannot produce the typical cramps and paralysis found in animals taken from collapsing populations (see Chitty, 1959, for a detailed discussion).

The extensive studies of Norway rats conducted by Barnett *et al.* (Barnett, 1958; Barnett *et al.*, 1960; Evans and Barnett, 1965) also contradict the suggestion that hypoglycaemia plays a significant role in causing death in social stress situations. These authors found indications in subordinate Norway rats of considerable increase in adrenocortical activity, inhibition of thyroid function, and a reduction in glycogen content of the liver. However, even dying animals had a normal, or even slightly elevated, blood sugar level. None of the observed changes can explain the resulting deaths (Barnett, 1964).

b) Renal Function

The kidneys of subordinate tree-shrews correspond histologically to the "shock kidneys" observed in human beings after serious loss of blood or following therapy with powerful blood-pressure reducing drugs (Zollinger, 1966). However, in the case of tree-shrews, a general drop in blood-pressure is improbable, since this is usually accompanied by a fall in skin temperature. In subordinate tree-shrews, however, skin temperatures can

be 1–2° C above the normal value, with a considerable drop occurring just before death. Collection of blood from the tail arteries also showed no indication of a blood-pressure drop—at least not to the extent that would be necessary to explain complete arrest of renal circulation. The deficient renal blood flow is thus most unlikely to be a consequence of an overall drop in the animal's blood-pressure, but is probably due to specific constriction of renal blood vessels.

Deleterious effects upon renal function can arise from increased adrenocortical activity as well as from high activation of the sympathetic nervous system and release of adrenaline from the adrenal medulla. These factors will now be considered in detail.

Influence of Adrenal Cortex. The adrenocortical hormones released into the blood in response to stressors of various kinds can provoke renal damage. Selye and Constantinides (1948) found that, following treatment with corticoid hormones, their experimental animals exhibited renal lesions—nephrosis and sometimes even acute nephritis. This finding has since been repeatedly confirmed (see Zollinger, 1966). For instance, stimulation of adrenocortical activity in mice by daily administration of ACTH leads, after only 20 days, to pronounced dilation of individual glomerular capillaries and to lesions. The renal tubules become expanded, and necrosis reminiscent of thrombosis appears in the renal medulla (Christian, 1964a). Even natural activation of adrenal function by cold stress leads to severe kidney damage particularly in the renal tubules (rats: Munday and Blane, 1961).

In contrast to activation of the sympathetic nervous system (see below) adrenocortical hormones do not lead to renal ischaemia, and, in addition, the kidneys usually become hypertrophied (Constantinides, 1951; Gross and Meier, 1951; Pasley and Christian, 1971).

The causes of the pathological effects of increased adrenocortical activity are as yet obscure. Some authors (Skelton, 1955; Anderson, 1963) assume that there is direct damage to the glomerular tufts caused by corticoid hormones; but it seems more likely that there is secondary deterioration of the renal vessels brought about by the action of hormones on the mineral balance of the body, and thus upon blood pressure (see Zollinger, 1966).

Influence of Sympathetic Nervous System. The kidney has a very rich supply of vasoconstrictor fibres from the thoracolumbar region. Electrical stimulation of these sympathetic fibres produces, in all animals so far investigated (rat, rabbit, dog, cat), immediate renal ischaemia, which is primarily due to vasoconstriction of afferent arterioles[3]. After only a few seconds, the renal tubules become collapsed; the production of urine is

3 Study and Shipley, 1950; Houck, 1951a; Block *et al.*, 1952; Cort, 1953; Kubicek *et al.*, 1953; Eichholtz *et al.*, 1954.

at once restricted. However, it has not been experimentally possible to produce persistent renal ischaemia or anuria by continuous stimulation of renal nerve fibres (Block *et al.*, 1952).

Similar renal vasoconstriction can be produced by stimulating vasomotor centers in the brain. Thus, Green and Hoff (1937) found in cats and monkeys that kidney volume diminished during electrical stimulation of the cerebral cortex (sigmoid gyrus). In line with the data presented below, this indicates vasoconstriction in the kidney. In the dog, electrical stimulation of various areas in the brain-stem leads to pronounced restriction of renal blood flow and urine production (Wise and Ganong, 1960). Steinmetz and Kiley (1960) found that patients with lesions in the frontal cortex area had acute renal failure, which they interpreted as a result of renal vasoconstriction.

In the present context, the results of Hoff *et al.* (1951) with cats are of particular interest: Electrical stimulation of narrowly defined areas in the sigmoid gyrus produces immediate ischaemia in the renal cortex. Experiments in which brief stimulation was given several times, over a period of several days or weeks, led to pathological changes in the kidneys, which correspond to "lower nephron nephrosis". Kidneys with this condition resemble, in their histological appearance, the human "shock kidney", and many authors have therefore used this same term to describe them.

A strong influence exerted on renal function by catecholamines released with any pronounced activation of the sympathetic nervous system was described by Unna as early as 1935, and has since been confirmed by many ther authors. Release of adrenaline restricts renal circulation in proportion to the dose, and there is a corresponding change in glomerular filtration and in urine production[4].

The response of individual nephrons or their blood vessels to adrenaline administration is not uniform. Whereas circulation in some regions of the kidney returns to normal even with a continuous supply of adrenaline, other areas remain ischaemic for quite a long time after cessation of the administration of vasopressor substances (Moses, 1952; Epstein *et al.*, 1970). Variation in the responsiveness of individual renal vessels could explain why individual, presumably functional, glomeruli can still be found in tree-shrews with kidneys showing widespread ischaemia.

The volume (or weight) of kidneys decreases in proportion to the dose following injection with adrenaline (Shultz, 1970), and can thus be taken as an index of the filling stage of the kidneys (see also Collier and Swann, 1971). Therefore, the decrease in kidney weight observed in subordinate tree-shrews could depend to some extent on renal vasoconstriction.

4 Houck, 1951b; Moses, 1952; Mills *et al.*, 1953; Carpenter and Kunin, 1961; Ogle, 1968; Shultz, 1970; Epstein *et al.*, 1970.

It would seem that no experiments involving investigation of the effects of long-term administration of high doses of adrenaline on kidney function have yet been conducted. However, in human beings with tumours in the adrenal medulla ("phaeochromocytoma"), varying degrees of disruption of renal function occur (uraemia, oliguria or anuria). The renal tubules are dilated, and in many cases there is widespread necrosis in the renal medulla (Carpenter and Kunin, 1961; Zollinger, 1966). Such damage is interpreted as the result of restriction of renal blood flow by periodically or permanently raised adrenaline levels in the blood.

Influence of Stressful Situations. The results cited so far were almost exclusively derived from anaesthetised animals. They show that activation of the sympathetic nervous system can lead to impairment of renal function through renal nerves as well as through adrenaline. In a wakeful state, as well, a decrease in renal blood flow occurs as part of the reactions of an organism against stressful stimuli. Thus in 1859, Bernard described oliguria in laboratory animals and man following pronounced pain or strong emotional responses. This finding has since been confirmed by many authors (summarised by Mason *et al.*, 1968). However, it is not yet clear how far the effects on urine production and release were elicited by nervous and/or hormonal influences (adrenaline, vasopressin, etc.). In man, Wolf *et al.* (1948) showed that pain and psychological stress are accompanied by acute renal vasoconstriction. Meehan (1960) determined the renal plasma flow of human subjects who had been induced to adopt various emotional states (such as "happiness, fear and depression") under hypnosis. All these different kinds of emotion as well as exposure to cold or severe physical exertion, led to a reduction in renal circulation—a particularly pronounced fall (by about 50%) ocurring with "depression".

Conclusions. As has been shown by the findings presented above, ergotropic situations involving a rise in blood-pressure are particularly characterized by a decrease in renal blood flow. Thus, the kidney responds like the rest of the "splanchnic" viscera; its blood flow is preferentially shunted to skeletal muscle (Pickering, 1968; Forsyth and Harris, 1970; Collier and Swann, 1971). Accordingly, a typical picture of renal ischaemia can occur without loss of blood and with constant, or even increasing, blood-pressure.

With regard to tree-shrews I am inclined to draw the following conclusions from the above and from the results reported here: Confrontation with the victor leads in some tree-shrews to immediate and total renal failure. This can be explained as the result of sympathetic arousal. In contrast to the situation of artificial stimulation of the renal nerves (see p. 265), the renal circulation can apparently be reduced for a period of days in such cases.

It seems unlikely, however, that this sustained renal vasoconstriction is caused exclusively by the sympathetic nervous system. Possibly the primary decreased renal blood flow results in an increase of angiotensin level, which would maintain or even potentiate renal vascoconstriction.

In some subordinate tree-shrews, renal function is initially adequate to ensure excretion of toxic substances from the body. But even in these animals, the urea-nitrogen content of the blood begins to rise after 6–10 days of stress exposure, and death follows in a few days. This cannot be entirely explained (if at all) on the basis of high sympathetic nervous system activity. It indicates instead (additional?) damaging alterations in the mineral balance or in metabolism, which only take effect after some period of stress. Increased adrenocortical activity, which has been demonstrated in tree-shrews, is particularly significant in this respect. It is also possible that changes in blood pressure play an important role (e.g. via the renin-angiotensin mechanism). For example, Henry *et al.*[5] found in laboratory mice that there was always a considerable rise in blood-pressure and the concomitant appearance of severe pathological changes in the kidneys when the animals were exposed to long periods of social confrontation.

Thus, the origin of renal damage in subordinate tree-shrews is only understood to a limited extent; but further investigations should provide answers to some of the questions raised.

c) Indications of Renal Damage under Natural Conditions

Does impairment in renal function occur in free-living animals as well, and could this (perhaps in combination with other factors) provide an explanation for population crashes or for the death of subordinate animals? As yet, the function and/or morphology of the kidneys has only been considered in very few investigations. Nevertheless, a number of findings suggest possible kidney damage brought about by social stress.

Snowshoe Hare. The most comprehensive studies of physiological changes in natural populations have been carried out by Green *et al.* with snowshoe hares (*Lepus americanus*). The authors suggest that the cause of population crashes is hypoglycaemic shock; but various inconsistencies render this unlikely (see p. 263). Some of the data provided indicate, on the contrary, that there is severe renal damage in the animals of collapsing populations. For example, the kidneys of roughly 60% of the animals investigated showed advanced pathological changes (haemorrhage, tubular degeneration, etc.). The nonprotein nitrogen content in the blood of dying hares ($n = 21$) had a mean value of 89.2 (57.4–156.8) mg/100 ml, whilst with 10 animals examined prior to emergence of

5 Henry *et al.*, 1967; Henry and Cassels, 1969; Henry and Stephens, 1969; Henry *et al.*, 1971.

symptoms indicating impending death, the nonprotein nitrogen content was 49.8 (39.2–60.0) mg/100 ml. There was no investigation of control animals from normal (non-collapsing) populations; but these results alone indicate uraemia, though this is not discussed by the authors themselves.

Field Vole. In voles (*Microtus*), as well, indications of hypoglycaemia are not convincing (see p. 263). Chitty (1959) therefore rejected shock disease as cause of death. Animals from overcrowded populations exhibited anaemia and splenic hypertrophy (Dawson, 1956; Chitty, 1957). This led Chitty to the conclusion that haemolytic anaemia was the cause of population crashes. This anaemia, however, could also be a consequence of renal insufficiency (see p. 253). The symptoms preceding death as described by Frank (1953), i.e. convulsion and paralysis, and the pronounced fall in skin temperature, were also explicable on this basis. Studies of the function or morphology of vole kidneys have yet to be carried out. However, Andrews and Strohbehn (1971) found in males of the closely-related lemming (*Lemmus trimucronatus*) that there was cardiac hypertrophy and histologically demonstrable renal damage at times of high population density.

Laboratory Mouse. If male mice are exposed to the influence of daily confrontations with rivals for a period of weeks, conspicuous renal damage results (Henry *et al.*, see p. 267), though it is not severe enough to bring about the death of the animals.

The kidneys of males kept in groups for weeks were significantly lighter (by about 10%) than those of isolated controls (Welch and Welch, 1969).

The kidney weight of female mice which were housed for 6 weeks at high density and which exhibited no fighting behaviour was 12% ($p < 0.001$) below that of controls (Deisz, 1972).

Woodchuck and Sika Deer. Christian and his co-workers found pronounced kidney disease in woodchucks (*Marmota monax*) from overcrowded populations (Christian, 1964b) and in sika deer (*Cervus nippon*) from a collapsing population (Christian *et al.*, 1960). The pathological changes corresponded to glomerulonephritis of such intensity that in some animals it resulted in destruction of all glomeruli. The extent of renal disease was clearly correlated with population density; it was most pronounced in collapsing populations and ceased to appear after the number of individuals had been reduced. However, the authors did not regard renal disease as the factor responsible for the mass deaths.

Man. An impressive example of severe renal disease under extreme stress is known from man under the name of "war nephritis (Feldnephritis)". This epidemic disease—which corresponds to glomerulonephritis—was first observed in the American Civil War, in which it claimed many victims. It was equally widespread in the First and

Second World Wars, but the mortality rate was vastly reduced by means of improved treatment techniques. Early investigators attributed such renal damage to infectious germs, but this suggestion proved to be just as difficult to confirm as did various other hypotheses which were put forward: renal vasoconstriction through cold and dampness; poisoning by louse powder; damage to the brainstem (diencephalosis); allergically induced infections. Even today, the aetiology of war nephritis is completely obscure, and there is a whole range of mutally opposed hypotheses (Hoff, 1962; Brock, 1964; Zollinger, 1966; Doerr, 1970).

Our findings with tree-shrews suggest another possibility: Vasoconstriction of the renal vessels brought about by severe nervous tension and possibly magnified by cold or other environmental influences. Subsequent and additional damage to the glomerular capillaries through readily available germs (e.g. streptococci) would be more than likely with reduced or arrested renal blood flow.

The findings from natural populations suggest that social stress can lead to renal disease, though it is as yet unclear how far such renal damage can lead to malfunction of an organism or even death in the various animal species. The possibility seems to exist to date since renal function can be easily disrupted in all of the animals investigated—and in human beings as well—by brief activation of the sympathetic nervous system. However, apart from this study of tree-shrews, there are no further reports of long-term experimental studies on the influence exerted upon the kidney by social stress or by emotionally induced activation. Previous experiments have been exclusively concerned with renal damage provoked by toxic substances, crushing of muscles, ligation of renal vessels, etc. It would seem that the participation of persistent emotional processes in the origin of renal damage has been generally neglected. This is surprising, since renal diseases—particularly those associated with elevated blood-pressure—have a prominent place among civilized ailments and are gradually increasing in frequency without any obvious cause having been established.

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References

Anderson, M. S.: Electron microscopy of the glomerulus and renal tubules in experimental hypertension. Amer. J. Path. **43**, 257–272 (1963).

Andrews, R. V., Strohbehn, R.: Endocrine adjustments in a wild lemming population during the 1969 summer season. Comp. biochem. Physiol. **38** A, 183–201 (1971).

Andrzejewski, R., Petrusewics, K., Walkowa, W.: Absorption of newcomers by a population of white mice. Ekol. Polska, Ser. A **11**, 223–240 (1963).

Autrum, H., v. Holst, D.: Sozialer „Stress" bei Tupajas (*Tupaia glis*) und seine Wirkung auf Wachstum, Körpergewicht und Fortpflanzung. Z. vergl. Physiol. **58**, 347–355 (1968).

Baker, B. L.: A comparison of the histological changes induced by experimental hyperadrenocorticalism and inanition. Recent Progr. Hormone Res. **7**, 331–373 (1952).

Barnett, S. A.: Physiological effects of "social stress" in wild rats. 1. The adrenal cortex. J. psychosom. Res. **3**, 1–11 (1958).

Barnett, S. A.: Social stress. The concept of stress. In: Viewpoints in biology, vol. 3 (eds. J. D. Carthy and C. L. Duddington), p. 170–218. London: Butterworths 1964.

Barnett, S. A., Eaton, J. C., McCallum, H. M.: Physiological effects of "social stress" in wild rats. 2. Liver glycogen and blood glucose. J. psychosom. Res. **4**, 251–260 (1960).

Bernard, C.: Leçon sur les proprietés physiologiques et les altérations pathologiques des liquides de l'organisme. Vol. 1. Paris: Bailliere 1859.

Block, M. A., Wakim, K. G., Mann, F. C.: Circulation through kidney during stimulation of the renal nerves. Amer. J. Physiol. **169**, 659–669 (1952).

Brock, J.: Die Nieren. Berlin: VEV Verlag 1964.

Bronson, F. H., Eleftheriou, B. E.: Adrenal response to fighting in mice: separation of physical and psychological causes. Science **147**, 627–628 (1965).

Calhoun, J. B.: Mortality and movement of brown rats in artificially supersaturated populations. J. Wildl. Mgmt. **12**, 167–171 (1948).

Calhoun, J. B.: Space and the strategy of life. In: Behavior and environment (ed. A. H. Essor), p. 329–387. New York-London: Plenum Press 1971.

Carpenter, A. A., Kunin, A. S.: Pheochromocytoma with acute tubular necrosis. New Engl. J. Med. **265**, 986–988 (1961).

Chitty, D.: Self-regulation of numbers through changes in viability. Cold Spr. Harb. Symp. quant. Biol. **22**, 277–280 (1957).

Chitty, D.: A note on shock disease. Ecology **40**, 728–731 (1959).

Christian, J. J.: The adreno-pituitary system and population cycles in mammals. J. Mammal. **31**, 247–259 (1950).

Christian, J. J.: Endocrine adaptive mechanisms and the physiologic regulation of population growth. In: Physiological mammalogy, vol. 1, Mammalian populations (eds. W. V. Mayer and R. G. van Gelder), p. 189–353. New York-London: Academic Press 1963.

Christian, J. J.: Glomerular renal disease in mice after chronic ACTH treatment. J. Endocr. **28**, 6–7 (1964a).

Christian, J. J.: Physiological and pathological correlates of population density. Proc. roy. Soc. Med. **57**, 169–174 (1964b).

Christian, J. J., Flyger, V., Davis, D. E.: Factors in the mass mortality of a herd of Sika deer, *Cervus nippon*. Chesapeake Sci. **1**, 79–95 (1960).

Collier, R. O., Swann, H. G.: Relation of kidney size to blood pressure. Amer. J. Physiol. **220**, 488–491 (1971).

Constantinides, P.: An immediate kidney response to acute stress. Endocrinology **49**, 512–521 (1951).

Cort, J. H.: Effect of nervous stimulation on the arteriovenous oxygen and carbon dioxide differences across the kidney. Nature (Lond.) **171**, 784–785 (1953).

Dawson, J.: Splenic hypertrophy in voles. Nature (Lond.) **178**, 1183–1184 (1956).

Deisz, R.: Unpublished data: Zoologisches Institut der Universität. München 1972.

Doerr, W.: Spezielle pathologische Anatomie I. Berlin-Heidelberg-New York: Springer 1970.

Dugle, J. B., Free, A. H.: A simple blood test for the assessment of renal function in experimental animals. Lab. Anim. Care **19**, 624–628 (1969).

Eichholtz, F., Taugner, R., Braun, W.: Untersuchungen zur Behandlung renaler Ischämien. Arch. int. Pharmacodyn. **98**, 118–128 (1954).

Elton, R. L., Zarrow, I. G., Zarrow, M. X.: Depletion of adrenal ascorbic acid and cholesterol: a comparative study. Endocrinology **65**, 152–157 (1959).

Epstein, M., Hollenberg, K., Merrill, J. P.: The pattern of the renal vascular response to epinephrine in man. Proc. Soc. exp. Biol. (N.Y.) **134**, 720–724 (1970).

Erslev, A. J.: Anemia of chronic renal disease. Arch. intern. Med. **126**, 775–780 (1970).

Evans, C. S., Barnett, S. A.: Physiological effects of "social stress" in wild rats. 3. Thyroid. Neuroendocrinology **1**, 113–120 (1965/66).

Forsyth, R. P., Harris, R. E.: Circulatory changes during stressful stimuli in rhesus monkeys. Circulat. Res. **26**, Suppl. 1, 13–20 (1970).

Frank, F.: Untersuchungen über den Zusammenbruch von Feldmausplagen (*Microtus arvalis* Pallas). Zool. Jb. Abt. System, Ökol. u. Geogr. **82**, 95–136 (1953).

Green, H. D., Hoff, E. C.: Effects of faradic stimulation of the cerebral cortex on limb and renal volumes in the cat and monkey. Amer. J. Physiol. **118**, 641–658 (1937).

Green, R. G., Larson, C. L.: Shock disease of wild snowshoe rabbits. Amer. J. Physiol. **119**, 319–320 (1937).

Green, R. G., Larson, C. L.: A description of shock disease in the snowshoe hare. Amer. J. Hyg. **28**, 190–212 (1938).

Green, R. G., Larson, C. L., Bell, J. F.: Shock disease as the cause of the periodical decimation of the snowshoe hare. Amer. J. Hyg. **30** B, 83–102 (1939).

Gross, F., Meier, R.: Die Beeinflussung der kompensatorischen Nierenhypertrophie durch Cortisone und andere Nebennierenrindensteroide. Experientia (Basel) **7**, 74–76 (1951).

Henry, J. P., Cassel, J. C.: Psychosocial factors in essential hypertension. Recent epidemiologic and animal experimental evidence. Amer. J. Epidem. **90**, 171–200 (1969).

Henry, P., Ely, D. L., Stephens, P. M., Ratcliffe, H. L., Santisteban, G. A., Shapiro, A. P.: The role of psychosocial factors in the development of arteriosclerosis in CBA mice: observations on the heart, kidney and aorta. Artherosclerosis **14**, 203–218 (1971).

Henry, P., Meehan, J. P., Stephens, P. M.: The use of psychosocial stimuli to induce prolonged systolic hypertension in mice. Psychosom. Med. **29**, 408–432 (1967).

Henry, P., Stephens, P.: The use of psychosocial stimuli to induce renal and cardiovascular pathology in mice. Psychosom. Med. **31**, 454–455 (1969).

Hoff, E. C., Kell, J. F., Jr., Hastings, N., Sholes, D. M., Gray, E. H.: Vasomotor, cellular and functional changes produced in kidney by brain stimulation. J. Neurophysiol. **14**, 317–332 (1951).

Hoff, F.: Klinische Physiologie und Pathologie. Stuttgart: Thieme 1962.

Holst, D. v.: Sozialer Stress bei Tupajas (*Tupaia belangeri*). Die Aktivierung des sympathischen Nervensystems und ihre Beziehung zu hormonal ausgelösten

ethologischen und physiologischen Veränderungen. Z. vergl. Physiol. **63**, 1–58 (1969).

Holst, D. v.: Die Funktion der Nebennieren männlicher *Tupaia belangeri:* Nebennierengewicht, Ascorbinsäure und Glucocorticoide bei kurzem und bei andauerndem soziopsychischem Stress. J. comp. Physiol. **78**, 289–306 (1972).

Houck, C. R.: Alteration of renal hemodynamics and function in separate kidneys during stimulation of the renal artery nerves in dogs. Amer. J. Physiol. **167**, 523–530 (1951a).

Houck, C. R.: Alterations in renal hemodynamics and function during intravenous injection of epinephrine in the dog. Amer. J. Physiol. **166**, 649–657 (1951b).

Kubicek, W. G., Kottke, F. J., Laker, D. J., Visscher, M. B.: Renal function during arterial hypertension produced by chronic splanchnic nerve stimulation in the dog. Amer. J. Physiol. **174**, 397–400 (1953).

Levi, L.: Sympatho-adrenomedullary responses to emotional stimuli: methodologic, physiologic, and pathologic considerations. In: An introduction to clinical neuroendocrinology (ed. E. Bajusz), p. 78–105. Basel-New York: Karger 1967.

Mason, J. W.: A review of psychoendocrine research on the sympathetic-adrenal medullary system. Psychosom. Med. **30**, 631–653 (1968).

Mason, J. W., Jones, J. A., Ricketts, P. T., Brady, J. V., Tolliver, G. A.: Urinary aldosterone and urine volume responses to 72-hr. avoidance sessions in the monkey. Psychosom. Med. **30**, 733–745 (1968).

Meehan, J. P.: Central nervous control of the renal circulation. Amer. Heart J. **6**, 318–320 (1960).

Mills, L. C., Moyer, J. H., Skelton, J. M.: The effect of norepinephrine and epinephrine on renal hemodynamics. Amer. J. med. Sci. **226**, 653–663 (1953).

Moses, J. B.: The distribution of renal ischemia produced by epinephrine and norepinephrine as demonstrated by the fluorescent dye vasoflavine. J. Urol. (Baltimore) **68**, 558–567 (1952).

Munday, K. A., Blane, G. F.: Cold stress of the mammal, bird, and reptile. Comp. Biochem. Physiol. **2**, 8–21 (1961).

Ogle, C. W.: Urinary output of rats in response to subcutaneous injections of adrenaline. Experientia (Basel) **24**, 496–497 (1968).

Pasley, J. N., Christian, J. J.: Effects of ACTH on voles (*Microtus pennsylvanicus*) related to reproductive function and renal disease. Proc. Soc. exp. Biol. (N.Y.) **137**, 268–272 (1971).

Penington, D. G., Kincaid-Smith, P.: Anaemia in renal failure. Brit. med. Bull. **27**, 136–141 (1971).

Pfleiderer, G.: Glykogen. Bestimmung als D-Glucose mit Hexokinase, Pyruvatkinase und Lactat-Dehydrogenase. In: Methoden der enzymatischen Analyse (Hrsg. H. Bergmeyer), S. 59–62. Weinheim: Verlag Chemie 1962.

Pickering, G. W.: High blood pressure. New York: Grune & Stratton 1968.

Raab, A.: Der Serotoninstoffwechsel in einzelnen Hirnteilen vom Tupaia (*Tupaia belangeri*) bei soziopsychischem Stress. Z. vergl. Physiol. **72**, 54–66 (1971).

Reimer, J. D., Petras, M. L.: Breeding structure of the house mouse, *Mus musculus*, in a population cage. J. Mammal. **48**, 88–99 (1967).

Richter, C. P.: On the phenomenon of sudden death in animals and man. Psychosom. Med. **19**, 191–198 (1957).

Selye, H.: The physiology and pathology of exposure to stress. Montreal: Acta 1950.

Selye, H., Constantinides, P.: Das Allgemeine Anpassungssyndrom und die Anpassungskrankheiten. Dtsch. med. Rdsch. **5**, 161–169 (1948).

Shultz, M. L.: Acute changes in renal size in normal and hypertensive dogs. J. Urol. (Baltimore) **104**, 629–634 (1970).

Skelton, F. R.: Experimental hypertensive vascular disease in the rat. Arch. Path. **60**, 190–200 (1955).

Sprankel, H.: Über Verhaltensweisen und Zucht von *Tupaia glis* (Diard, 1820) in Gefangenschaft. Z. wiss. Zool. **165**, 186–220 (1961).

Steinmetz, P. R., Kiley, J. E.: Renal tubular necrosis following lesions of the brain. Amer. J. Physiol. **29**, 268–276 (1960).

Study, R. S., Shipley, R. E.: Comparison of direct with indirect renal blood flow, extraction of inulin and diodrast before and during acute renal nerve stimulation. Amer. J. Physiol. **163**, 442–453 (1950).

Takaku, F., Chiba, S., Nakao, K.: Erythropoietic activity of kidney extracts. Israel J. med. Sci. **7**, 972–976 (1971).

Unna, K.: Arterieller Druck und Nierendurchblutung. Pflügers Arch. ges. Physiol. **235**, 515–519 (1935).

Vecsei, P., Csalay, L.: Vergleichende Untersuchungen über die Corticosteroidbildung und das morphologische Verhalten der Nebennieren bei chronischen Reizen ausgesetzten Tieren. Z. Vitaminforsch. **14**, 57–65 (1965).

Walther, D.: Das Substrat der Anurie und Polyurie beim akuten Nierenversagen der Ratten. In: Normale und pathologische Funktionen des Nierentubulus (Hrsg. K. J. Ullrich und K. Hierholzer), S. 291–297. Bern-Stuttgart: Hans Huber 1965.

Welch, B. L.: Psychophysiological response to the mean level of environmental stimulation: a theory of environmental integration. Symp. Med. Asp. Stress in Milit. Climate, p. 39–99, 1964.

Welch, B. L., Welch, A. S.: Sustained effects of brief daily stress (fighting) upon brain and adrenal catecholamines and adrenal, spleen, and heart weights in mice. Proc. nat. Acad. Sci. (Wash.) **64**, 100–107 (1969).

Wise, B. L., Ganong, W. F.: Effect of brain-stem stimulation on renal function. Amer. J. Physiol. **198**, 1291–1295 (1960).

Wolf, S., Pfeiffer, J. B., Ripley, H. S., Winter, O. S., Wolf, H. G.: Hypertension as a reaction pattern to stress; summary of experimental data on variations in blood pressure and renal blood flow. Ann. intern. Med. **29**, 1056–1076 (1948).

Wynne-Edwards, V. C.: Animal dispersion in relation to social behaviour. Edinburgh-London: Oliver & Boyd 1962.

Zollinger, H. U.: Niere und ableitende Harnwege. Berlin-Heidelberg-New York: Springer 1966.

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