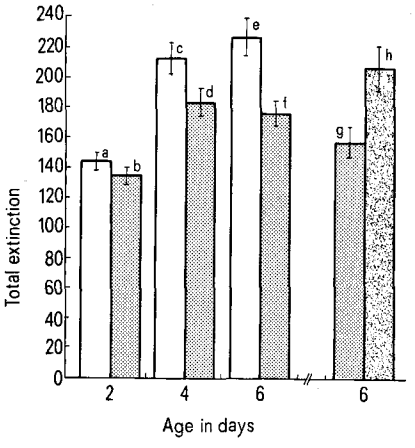
with 20 µg of JH III (Calbiochem, San Diego, California) or acetone. 24 h later the brains from these insects were processed as described before. 5 µm thick paraffin sections were stained with PF and the amount of PF-stainable material in the A-cell perikaryon was determined microspectrophotometrically[8,9] by using a scanning microscope photometer (Carl Zeiss, Oberkochen, W. Germany) linked to a PDP-12 computer. The measuring apperture was set at 1.6 µm and extinction measurements were taken at 490 nm and 1 µm step size from sections of 15–30 A-cells, from a minimum of 6 insects, for each group. The data were analysed using 1 way analysis of variance or by the t-test.



Amount of PF-stainable material (total extinction) in the neurosecretory A-cells. a, c and e acetone treated control; b, d and f treated with 50 µg precocene II on day 0; g treated with precocene II, by contact method, on day 0, and 2 µl acetone on day 5; h treated with precocene II, by contact method, on day 0, and 20 µg JH III on day 5. Vertical bars represent twice the SE of the mean.

Results and discussion. In insects treated with precocene alone there was no sign of yolk deposition in the oocytes, whereas application of JH III to precocene-treated insects induced yolk deposition. Analysis of the data obtained by microspectrophotometry (figure) revealed that in precocene-treated insects there was a significant reduction in the amount of stainable material 4 and 6 days after the treatment ($p<0.05$; $p<0.01$). There is no evidence to suggest that this decrease is due to an accelerated rate of transport of neurosecretory material from the perikaryon to the neurohaemal site in the aorta. The decrease is probably due to an inhibition in the synthetic activity of the cells. The dynamics of the A-cells in the control insects is comparable to that of normal insects[9]. Within 24 h after JH-treatment of precocene-treated insects, there is a significant increase in the amount of PF-stainable material in the A-cells ($p<0.01$) (figure), indicating an apparent stimulatory effect. Therefore, it is probable that the inhibition of these cells after precocene-treatment may be due to the absence of a positive feed back from the CA. It is pertinent to mention that injection of JH or implantation of CA into allatectomized *Schistocerca gregaria* stimulates the synthetic activity of the A-cells[10,11]. From the present studies it is not known whether the inhibition and subsequent degeneration of the CA, after precocene-treatment[5,6] is mediated through the A-cells, although removal of these cells leads to subnormal size of the CA[7].

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PRO EXPERIMENTIS

Blood volume and extracellular space (ECS) of the whole body and some organs of the rat

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Summary. Methods are described for estimation of blood volume and extracellular space (ECS) in the whole body and in some organs with ^{51}Cr, ^{14}C-thiocyanate and ^{3}H-inulin. A mean blood volume of 47 ml/kg, a thiocyanate space of 350 ml/kg and a inulin space of 288 ml/kg were determined in the rat. The corresponding values of organs are shown in figures 1–3.

Blood volume and extracellular space of rats have already been determined by several authors[1–3]. The total blood volume or the blood content of the organs can be determined either by volume of plasma with Evans Blue (T 1824) and ^{131}J marked albumin, or by the volume of erythrocytes with ^{32}P, ^{55}Fe or ^{51}Cr. If ^{51}Cr is used as tracer, the isotope is absorbed by the erythrocytes and excreted by the kidneys to a very small degree[4], because ^{51}Cr effectively combines with the globins components of the haemoglobines[5]. The object of this paper was to determine the blood volume and the extracellular space in the total animal and in organs as exactly as possible. As we could not find any related data or other details, the point of interest of those experiments was the determination in the organs.

Methods. General. Adult male Sprague-Dawley rats (Zentralinstitut für Versuchstierkunde, Hannover), weighing 170–240 g were used in this investigation. They were fed with pellets (Altromin, Lage/Lippe) ad libitum, but were fasted for 18 h prior to each experiment.

Red cell volume determination. ^{51}Cr was obtained from the Radiochemical Centre Amersham in form of $Na_2{}^{51}CrO_4$. After having sampled the blood with a heparinised capilette, a slight centrifugation is executed, because the ^{51}Cr-absorption in a saline erythrozyte-suspension is more complete than in pure blood[6,7]. The erythrocyte sediment is suspended in a physiological saline to a volume of 1 ml. After having added 15 µCi ^{51}Cr, the mixture was stored for 45 min at room temperature. The incubation was terminated by centrifugation, the erythrocytes were washed twice with 0.9% NaCl solution, the plasma was added again and 80 µl of reconstituted blood were injected into the tail vein. 50 µl of the suspension was applied as standard for the measurements of radioactivity. Blood samples were taken 60 min p.a. for activity measurements and determination of haematocrit;

Blood volume and extracellular space

	Blood volume (ml/kg)		Blood volume in organs (µl/g organ) Liver	Kidney	Heart	Brain	Spleen		ECS (ml/kg)
Own results ^{51}Cr	47.4 ± 8	Own results ^{51}Cr	94 ± 17	46 ± 14	42 ± 21	8.7 ± 9	574 ± 313	Own results inulin	288 ± 21
Sharpe[3] $^{55}Fe/^{59}Fe$	49.5	Lewis[14] T 1824	178	278	–	–	481	Own results thiocyanate	350 ± 22
Berlin[1] ^{32}P	45.9	Caster[15] T 1824	99	92	60	11	86	Wilde[16] inulin	249
Huang[8] T1824/^{32}P	57.5							*Huang[8] thiocyanate*	330
								Manery[17] chloride	290
								Harrison[2] chloride	290

Values represent the mean ± SE of the mean of 8 rats.

organ samples were hydrolyzed for 3 h in 5 N KOH at 100 °C and measured by a gamma szintillation counter (FH 41 A and FH 90 A, Frieseke & Höpfner). The haematocrit was determined with a heparinised capilette by centrifugation at 1500 × g for 30 min and corrected by a factor of 0.96. For correction of the whole body haematocrit, the factor 0.996 was used[8,9] (estimation by own measurements).

Determination of thiocyanate space. 200 mg/kg sodium thiocyanate with an activity of 2 µCi were injected i.p. into animal. Sodium thiocyanate reached equilibrium in the extracellular fluid within 55–60 min and remained constant for at least 80 min. At this time a sample of each organ was taken, equivalents were burned in a sample oxidizer (Tri carb 306, Packard) and measured in a liquid szintillation counter (SL 30, Intertechnique).

Determination of inulin space. The animal was anesthetized with Evipan® (100 mg/kg), one of its femoral veins or one of its tail veins was cannulated. Approximately 3–7 µCi inulin (70 mg/kg in 0.9% NaCl) were infused (infusion pump, Braun, Melsungen) over a period of 8–10 h. Loss of tracer due to leakage of syringes and fluctuation of infusion velocity were taken into consideration for the calculations. After equal distribution of the inulin, the organs were prepared (see thiocyanate space determination).

Calculation. Red cell volume, blood volume and thiocyanate space see Vetter[10] and Huang[8].

a) Red cell volume in organs (ml) $= \frac{A_{org} \cdot V_b \cdot H}{A_{inj} \cdot 100}$

A_{org}: measured activity in the organ (Imp/min)
V_b: total blood volume
A_{inj}: amount of ^{51}Cr injected (Imp/ml)
H: haematocrit

b) Inulin space $= \frac{v}{y \cdot k_{10}}$ (1-exp-k_{10}t) (Gladtke[11])

y: blood concentration of inulin (dpm/ml)
v amount of inulin injected (dpm/duration of the test)
k_{10}: elimination constant[12,13]

Results and discussion. The total blood volume of the rat amounts to 47 ml/kg b.wt. Relatively high variabilities occur in heart, spleen and brain. For heart and brain, these deviations are based on inadequacies of method (injuries of blood vessels when opening the cranium or blood portions remaining in ventricules). As some of the erythrocytes are stored in the spleen and in certain circumstances can be released, it is to be expected that different and too high blood volumes will be found in this organ. By the thiocyanate space method, an ECS of 350 ml/kg b.wt can be calculated. 80 min after i.p. injection there was no activity of ^{14}C-metabolites in excrements and urine.

A lower ECS of 288 ml/kg results when inulin method has been used. The same can also be applied to the ECS determination of the organs (except liver). These results are shown in figures 1–3. The results achieved with the ^{51}Cr method are already corrected by different factors (venous haematocrit, body haematocrit) and take account of the inhomogenous distribution of cellular and plasmatic blood contents which is caused by different flux velocity of erythrocytes and plasma. As the table shows, very different results have been found with T 1824 and ^{51}Cr method. The thiocyanate method results in an approximately 15% larger distribution space than the inulin method. The effect may be connected with the small influx of the thiocyanate into the tissues. Our results for ECS in the whole body of the rat conform well with values published in the literature[2,17]. No corresponding values determined by other authors for the ECS portions in the organs could be found. The ECS determination using inulin represents the most correct method known at present, but is rarely used because of the necessity of perfusion. The thiocyanate method, however, gives slightly higher ECS but with the advantage of a rapid and exact method of determination of ECS.

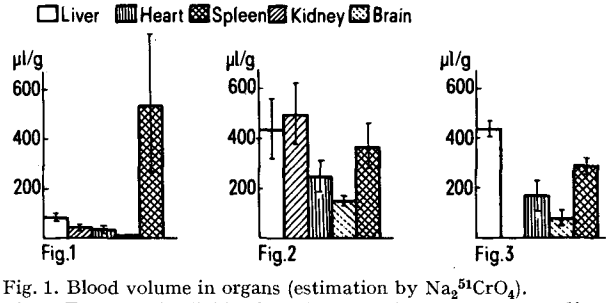

Fig. 1. Blood volume in organs (estimation by $Na_2{}^{51}CrO_4$).
Fig. 2. Extracellular fluid volume in organs (estimation by $NaS^{14}CN$)
Fig. 3. Extracellular fluid volume in organs (estimation by ^3H-inulin).

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