phase. The irregularities of the heat flux curve in Figure 2b obviously originate from the variable increase of cell mass. An interpretation of the results, together with a mathematical description, will be given in a later paper.

Irradiation device. UV-irradiation in the calorimeter is carried out by a low pressure mercury lamp (HNS 12, Osram/Berlin, maximal emission at 2537 Å) combined with a light guide of quartz glass (⌀ 10 mm, type QLG, Schott/Mainz). If the irradiation lamp is exactly focussed upon the surface of the fibres the intensity is 2.3 erg/mm^2. s measured by a photon flux detector (SCHAARSCHMIDT[8]). The radiant energy is partially converted into heat with a maximum value of 3 mcal after 10 min of irradiation. This additional heat may be diminished by compensation with a similar device placed in the opposite vessel, which requires a difficult and tedious adjustment of the lamp and the lenses. A heating resistor of 100 Ω proofed as an exact compensation unit if the values of current and voltage are arranged to a similar heat output. The irradiation device was used to record growth curves of radiation sensitive mutants of *Saccharomyces* during short intervals of UV-irradiation as well as heat flux measurements of resting cells in buffer. The changes of enthalpy in these thermograms are discussed in detail together with questions of repair processes elsewhere (SCHAARSCHMIDT[9]).

At the moment experiments with irradiation of visible light are being prepared to test the photoreactivation of radiation mutants of microorganisms and the photosynthesis of algae.

Zusammenfassung. Mit einer Lichtleiteranordnung können optische Messungen in einem Mikrokalorimeter E. CALVET durchgeführt werden. Eine weitere Anordnung ermöglicht Bestrahlungsversuche im Kalorimeter mit UV- und sichtbarem Licht.

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A New Method for Measuring Guanidine in Uremia

Due to lack of methods for the measurement of guanidine (G) in body fluids, no extensive studies are available on its serum and tissue concentrations and its renal excretion in normal and uremic subjects. In the present paper a procedure is described for measuring it in body fluids, and figures are reported concerning its serum levels and urinary excretion in normal persons and renal patients, its serum and muscle concentrations in normal and anuric dogs and its contents in certain widely used foods.

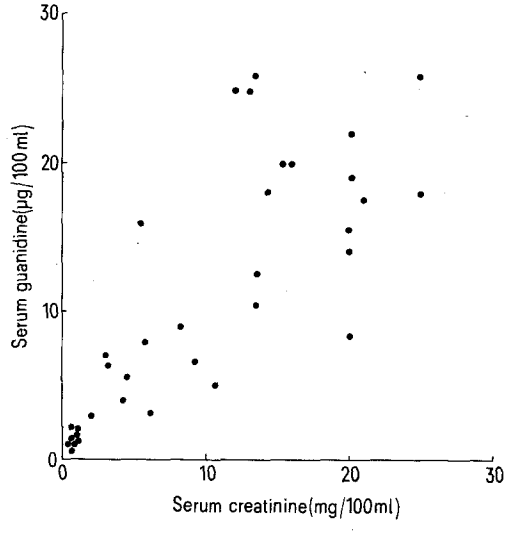
Materials and Methods. Normal serum (50 ml) and urine or uremic serum (25 ml) were diluted ten times with distilled water and passed through a column (6 × 20 mm) of Dowex W 50 resin (100–200 mesh) in the H+ form. The column was then washed with 100 ml of distilled water followed by 100 ml of 2*N* NH_4OH to elute creatine, creatinine (CR) and arginine. After a second water washing (100 ml) G and methylguanidine (MG) were eluted with 50 ml of 2*N* HCl. This was dried at 75 °C in a ventilated oven and the dry residue, dissolved in 50 ml distilled water, was passed through a column of Amberlite IRA 400 resin in the OH–form (1.5 × 28 cm), to remove Cl–. The eluate was dried again and the residue, quantitatively collected with 3 aliquots of 95% ethanol (2–3 ml each), pooled and reduced to exactly 1.0 ml. This solution was employed (in amounts from 0.2 to 0.5 ml) for chromatography on paper Watmann No. 1 with the system: ethanol: NH_3: water (90:5:5). After about 12 h descending migration at room temperature, G was satisfactorily separated from MG: Rf 0.39 and 0.47, respectively. The paper strips (55 × 5 cm) were dried in an air current and then sprayed with a mixture (2/3) of reagent No. 1 (diacetyl in water, 0.06%) and No. 2 (freshly prepared α–naphtol 1.0 g dissolved in 100 ml water containing g

Serum and muscle concentrations of creatinine, methylguanidine and guanidine in 2 normal dogs and in 7 dogs on the 3rd day of anuria following the ligature of the ureters

	Creatinine		Methylguanidine		Guanidine	
	Serum (mg/100 ml)	Muscle (mg/100 g)	Serum (mg/100 ml)	Muscle (mg/100 g)	Serum (μg/100 ml)	Muscle (μg/100 g)
Normal						
dog 1	0.60	10.0	0.008	0.06	0.8	3.2
dog 2	0.50	12.2	0.010	0.08	1.0	3.5
Anuric						
dog 1	11.8	19.1	0.12	0.29	10.0	14.0
dog 2	9.8	16.0	0.09	0.30	10.0	17.0
dog 3	19.0	21.0	0.19	0.34	5.5	20.6
dog 4	12.2	24.0	0.23	0.54	6.2	14.8
dog 5	14.0	22.0	0.08	0.20	9.1	28.3
dog 6	9.2	21.6	0.07	0.20	7.5	27.2
dog 7	6.6	11.4	0.05	0.17	10.6	30.3

6.0 NaOH and g 20 NaCl)[1]. The red spot of G was eluted from the paper with 5 ml of NH_3/aceton mixture (4/1) and read at 530 nm against a blank obtained by eluting an equal piece of sprayed paper where no colour was present. Recoveries of G added to normal and uremic serum and urine in amounts from 5 to 30 µg/100 ml, ranged from 76% to 94% (mean 84%) and the minimal amount which could be detected (by using 100 ml of fluid) was 0.5 µg. The measurements in muscle tissue and in solid foods were performed on the water extracts obtained with the procedure we had previously used for MG[2]. Eggs and milk were dialyzed in Visking cellophan bags against distilled water for 48 h at 4 °C and measurements were made on the dialyzate. CR was measured with an Auto-Analyzer and MG using the procedure previously described[3].

Results. The mean serum G concentration in 8 normal persons was 1.41 ± 0.49 µg/100 ml and its daily urinary excretion, 382 ± 221 µg. In 28 chronic renal patients the serum G levels increased in accordance with the severity of renal impairement, as indicated by the serum CR concentrations (Figure), while daily urinary output was 459 ± 229 µg, which is not significantly different from that of normal subjects. The serum G levels in 7 dogs on their 3rd day anuria following the legature of the ureters, was 8.4 ± 1.9 µg/100 ml and the content in their crued fresh muscle tissue was 21.0 ± 6.8 µg/100 g. The corresponding concentrations in 2 normal dogs were: 0.8–1.0 µg/100 ml and 3.2–3.5 µg/100 g, respectively (Table). The 2 vegetable foods (wheat meal and potatoes) examined did not contain appreciable amounts of G and the uncooked animal foods (milk, eggs, meat) were found to contain traces of it (less than 5 µg/100 g); boiled meat and broths on the contrary, contained amounts ranging from 20 to 50 µg/100 g or ml, respectively.



The relationship between the serum creatinine and the serum guanidine concentrations in 28 chronic renal patients ($r = 0.78$, $p > 0.01$) The figures concerning 8 normal subjects are also reported.

Discussion. The lack of specific colour reactions has been the main obstacle preventing G from being measured. We overcame this difficulty by using a combination of column and paper chromatography to isolate G from the other substances reacting to diacethyl. The procedure thus obtained, though requiring relatively large amounts of body fluids, is simple enough to be used in any clinical laboratory, and is absolutely specific. We can now state, therefore, that the accumulation of G in renal patients is in direct proportion to functional impairement and that it is preferentially retained in muscle tissue rather than in serum, as occurs for MG[2]. High serum G levels had been previously found in anuric dogs by CARR et al.[4] who employed, however, an unreliable procedure and, more recently in 7 renals patients, by STEIN et al.[5] who did not describe the analytical method employed.

The importance of G as an uremic toxin cannot be established on the grounds of the present results. It can only be pointed out that its accumulation inside the cells, together with MG, is likely to be responsible for enzymatic inhibitions. Indeed, it is known that both these substances produce such an effect in vitro[6].

As to the metabolic origin of G, its ingestion clearly cannot account for its urinary excretion even in the case of a diet rich in those foods which contain it in relatively large amounts (cooked meat, broth); it may therefore be concluded that the origin of G is largely endogenous.

Riassunto. Si descrive un metodo per la determinazione della guanidine nei liquidi biologici e si rileva che essa aumenta nel siero uremico fino a 30 volte la norma (valore medio normale: 1.41 ± 0.49 µg/100 ml) e che nei cani uremici la concentrazione muscolare è di quasi 3 volte superiore a quella plasmatica. La eliminazione urinaria non è diversa fra normali ed uremici: 382 ± 221 e 459 ± 229 µg/24 h, rispettivamente.

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Verkürzte Methode zur Isolierung radiochemisch reinen Chlorophylls

Hydrophobe Verunreinigungen (Wachse, Fette, Karotenoide und andere), die bei der spektrophotometrischen Bestimmung der Chrolophylle nicht stören, beeinflussen die Messungen ihrer Radioaktivität, da sie selbst radioaktiv sind. Sie wurden bisher meist durch mehrfaches Chromatographieren auf Papier entfernt (SCHLYCK[1]; GAPONENKO[2]). In der vorgelegten Arbeit wird eine verkürzte Methode zur semipräparativen Gewinnung radiochemisch reinen Chlorophylls *a* und *b* beschrieben.

Als Versuchsmaterial wurde Sommergerste (*Hordeum sativum* L.) der Sorte «Donaumarkt» (Slovensky dunajský trh) verwendet, im Dreiblattstadium. Radioaktiver ^{14}C-