obtain precise rate measurements at substrate concentrations between $K_m/10$ and $K_m/1$.

This criterion has not been met in reports now appearing in the literature.

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3. Supported in full by grant GM-10287-03 from NIH, PHS, Bethesda, Maryland.

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Helium-Glow Photometer for Picomole Analysis of Alkali Metals

Abstract. *The low-power electric-glow discharge in helium can excite effectively the characteristic emission lines of sodium and potassium. The helium-glow photometer used to generate the glow and measure the light is a relatively simple apparatus; it can be used to analyze samples containing 10^{-14} mole or more of sodium and potassium. Overall precision of the apparatus and method is 5 percent or better.*

Improved analytic techniques have permitted more thorough study of the function and performance of subunits of biological systems. For example, micropuncture techniques have permitted renal physiologists to study certain details of the process of urine formation; samples of nanoliter size obtained from renal tubules can be analyzed for their constituents. The total sodium and potassium in such samples is generally less than 10^{-10} mole, and special techniques are required to determine such quantities (*1*).

Operation of the helium-glow photometer (*2*) is based on the fact that electrical excitation of helium generates very energetic, metastable helium atoms that can transfer their energy to impurity atoms and excite them. Sodium or potassium atoms present in the glow will emit their characteristic radiation.

The physical layout of the photometer is sketched in Fig. 1. A pipette of the type described by Prager *et al.* (*3*), with which portions of a sample are placed on a wire electrode, is mounted on the 30× binocular microscope. The pipette mount provides three degrees of translational freedom so that the tip of the pipette can be positioned in the center of the field of the microscope; microscope and pipette move together during the procedure of placing the sample. Samples are held in an oil-filled trough mounted on a rack-and-pinion translating mechanism. The tip of the transfer pipette can be observed through a cover slip window on the side of the trough as the tip is lowered through the oil into a sample drop. The 10-watt radio-frequency (rf) supply is contained within the box that supports the discharge chamber, sample-trough support, and the two photomultiplier housings. A single-envelope twin tetrode is wired as an oscillator tuned to 27.12 Mhz. The output of the oscillator is applied between the upper ring electrode of Teflon-insulated wire and the lower electrode on which sample portions are placed. The lower electrode is an inverted "V" made of iridium wire 0.2 mm in diameter (*4*) and held in clips mounted through a Teflon disc on the oscillator enclosure (Fig. 2). The apex of the "V" is flattened with a fine abrasive stone to facilitate placement of portions of the sample. Line-frequency heating current from an adjustable supply is applied to the lower electrode to heat it and to volatilize the sample into the glow region.

Table 1. Compositions and experimentally determined sodium concentrations of nine artificial renal-tubule fluids. All fluids also contained the following concentrations: $(NH_2)_2CO$, 25 mg/100 ml; $C_6H_{12}O_6$, 50 mg/100 ml; KCl, 5m*M*; $NaHCO_3$, 25 m*M*.

$CaCl_2$ (mg/100ml)	$MgSO_4$ (m*M*)	NaH_2PO_4 (m*M*)	NaCl (m*M*)	Total (meq/lit.)	Na found (meq/lit.)
8	1	1	114	140	144
8	1	1	100	126	127.5
8	1	1	120	146	146.5
8	1	1	109	135	133.5
8	1	1	124	150	151.3
8	1	1	100	126	130.1
8	1	0	125	150	153
0	1	1	125	151	150.2
8	0	1	109	135	135

Table 2. Analyses of five nitric acid extracts of kidney tissue; comparison of results (meq/liter) by regular flame photometry (FP) and helium-glow photometry (HGP).

FP	HGP	Difference (%)
	Sodium	
287	288	+0.35
279	267	−4.30
285	286	+0.35
269	283	+5.21
	270	+0.37
	279	+3.71
264	272	+3.03
	286	+8.34
Mean difference (%) s.d.		+2.12 ± 3.82
	Potassium	
259	276	+6.56
260	244	−6.15
262	262	0.00
220	237	+7.73
	230	4.35
	232	5.55
225	219	−2.66
	218	−3.11
Mean difference (%) s.d.		1.62 ± 5.20

Helium flowing at a few cubic centimeters per second enters through an annular opening at the base of the chamber and flows out through a tube at the top. The effluent gas passes through a rotameter flowmeter and to the atmosphere through an oil-filled bubble trap that reduces the back diffusion of atmospheric gases. The chamber is sealed with an O-ring to the Teflon disc supporting the filament; the opening through which the transfer pipette is passed is sealed during use with another O-ring and a spring-loaded coverplate. The window at the front of the chamber is used to observe the delivery of a sample portion to the iridium wire; the window at the side of the chamber passes light from the discharge zone to the photometric system. These two windows are made of 25-mm diameter cover slips and are sealed to the chamber with a silicone rubber (*5*). A third window, made of Vycor, permits light from a 4-watt ultraviolet lamp (*6*) to fall on the iridium wire; the light insures that the glow starts uniformly.

A lens of short focal length collimates a portion of the light from the region of the discharge near the apex of the iridium "V"; the light passes to a dichroic mirror (*7*) that selectively reflects most of the yellow sodium light and transmits most of the near-infrared potassium light. Multilayer, all-dielectric interference filters (*8*) transmit the desired light to secondary lenses that spread the light over the photocathodes of the photo-

multipliers. Glass filters of suitable transmission characteristics are used to limit the phototube peak anode current to less than 10 μa. The typical filter for potassium is Corning color-specification No. 7-69; this is used with sample portions containing about 10^{-12} mole of potassium. The typical filter set for sodium includes Corning color-specification Nos. 2-62 and 4-64; this set has been used for samples containing about 4×10^{-11} mole of sodium. An adjustable, regulated, high-voltage supply is used for the photomultipliers; the supply can be adjusted between 500 and 1250 volts; the usual setting is around 800. The high-voltage and filter sets are adjusted to a suitable sensitivity. The effective excitation of the alkali-metal resonance radiation by the helium discharge permits the use of low-dark-current 1P21 photomultipliers for both the sodium and potassium systems. The quantum efficiency of the S-4 photosensitive surface is very low at 767 nm so that the 1P21 photomultiplier is not usually useful in ordinary flame photometers for potassium, but the sensitivity is adequate to permit detection of 10^{-14} mole of potassium with the helium-glow photometer.

The phototube anode currents are integrated during the discharge by two operational amplifier integrators (*9*). The output voltage of the integrators is displayed on a mirror-scale taut-band suspension meter (*10*); a selector switch connects the meter to either the potassium or the sodium integrator. Two semi-independent controls permit adjustment of the integration times of both systems. A set of monostable multivibrators controls the integration times and the period during which the iridium wire is heated and the rf voltage is applied to the electrodes. The integrators and multivibrators operate from common plus-and-minus 15-volt regulated power supplies.

Several elements present in biological fluids can influence the emission of sodium or potassium light, and there is a certain amount of mutual interaction between sodium and potassium. Several spectroscopic buffering materials were tried in order to find a sample diluent that would minimize these effects. The diluent chosen contains 5 m*M* $NH_4(H_2)PO_4$ and 30 m*M* $CsNO_3$; the phosphate reduces the effect of variable sample concentrations of phosphate, sulfate, chloride, and other suppressive agents, while the cesium enhances the light emission and

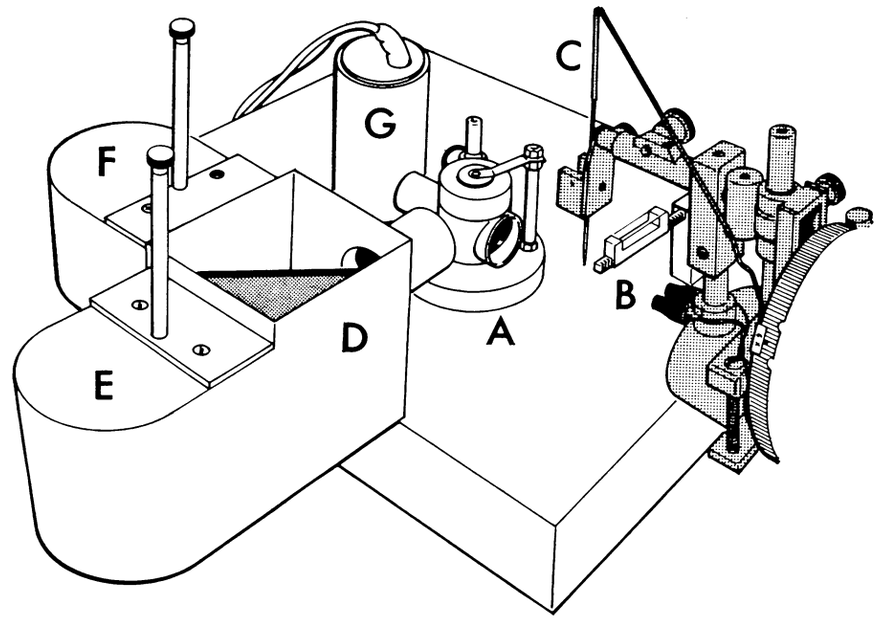

Fig. 1. Layout of the helium-glow photometer. *A*, Glow chamber; *B*, sample trough; *C*, transfer pipette; *D*, dichroic mirror housing (cover removed for clarity); *E*, potassium phototube; *F*, sodium phototube; *G*, ultraviolet lamp.

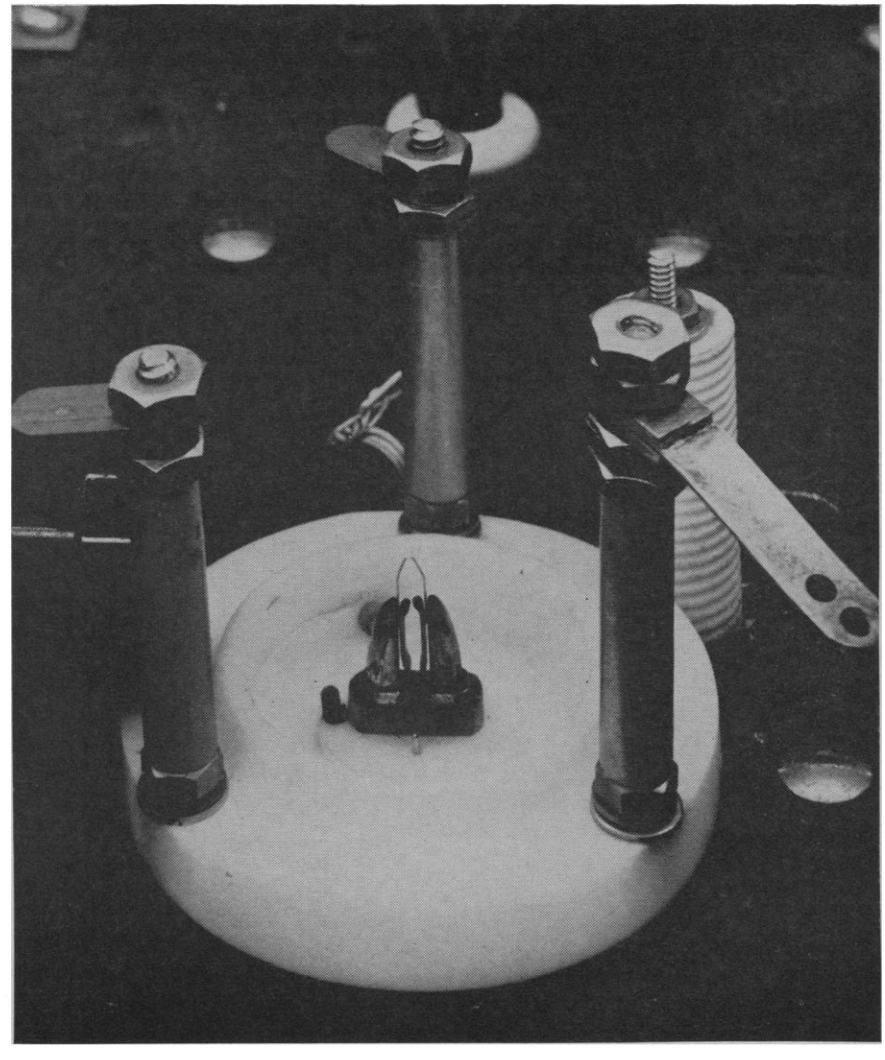

Fig. 2. Details of the lower electrode holder.

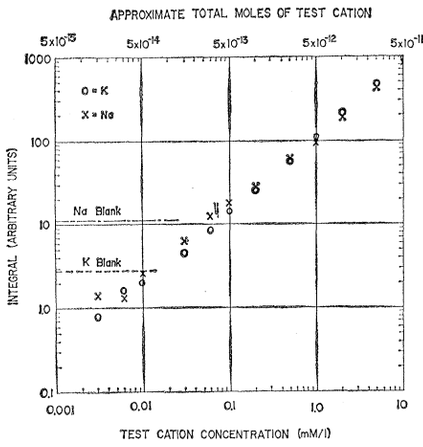

Fig. 3. Integrator responses (corrected for blank values) to various sodium and potassium contents of samples. Values in the upper scale are based on the estimated (within 20 percent) volume of the pipette used.

reduces interaction between sodium and potassium. Samples are diluted at least 20-fold with this diluent. Presence of sodium and potassium as impurities in the diluent can limit the sensitivity of the method. Figure 3 is a plot of the integral (in arbitrary units) of the sodium and potassium light over a four-decade range, showing results that can be obtained; the value for the blank was subtracted from each point. The volume of the pipette used for this run was estimated from its dimensions and was not determined precisely, so that the sample size shown on the top scale is approximate. Each point is the mean of readings for three sample portions; the spread between readings was less than 2 percent of the mean.

The analytic routine consists of several steps. Drops of tissue-extract fluid or tubule fluid diluted 20-fold, together with drops of standard composition, are placed under oil in the sample trough. Portions of the samples are transferred to the iridium wire, the chamber is closed, and atmospheric gases are purged from the chamber by the flowing helium. After purging for 2 minutes, the glow discharge between the iridium and ring electrodes is initiated by application of the rf field. Concurrently with the start of the glow, the iridium wire is heated electrically, and the sample is volatilized into the glow region. The light emitted is measured with the phototube integrator systems, the outputs of which are shown by the meter. After the meter readings are recorded, the glow and iridium heating currents are turned on again for 5 seconds to remove any sample residue. Each operational cycle requires about 3 minutes.

We made several samples of "artificial kidney-tubule fluid" and tested them for sodium content. Table 1 lists the compositions of the samples and the amount of sodium found in each; the potassium-sensing portion of the system was not used during this test. Portions of the diluted solutions were transferred to the iridium wire, and each solution was run three times. Agreement between the true and "found" values is within 3.5 percent.

To test the usefulness of the helium-glow photometer for determining sodium and potassium contents of tissue, five samples of kidney tissue were extracted with nitric acid. The extracts were approximately neutralized with NH_4OH, and a portion of each was analyzed for sodium and potassium on a Baird flame photometer. Another portion was diluted and tested with the helium-glow photometer as in the previous experiment. Table 2 compares the results by the two methods, which differed by not more than 8.4 percent; the difference was generally less than 5 percent.

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4. During development of the helium-glow photometer it was observed that sodium light was emitted from platinum electrodes after the electrodes were heated to incandescence; the effect was more pronounced if a sodium-free sample portion containing potassium was placed on the wire. This retention effect is also observed with tungsten, tantalum, and molybdenum. Pure iridium does not show the effect significantly.
5. Dow Corning, RTV 732.
6. Westinghouse type-794 Odorout.
7. Liberty Mirror Div., Libbey-Owens-Ford Glass Co., Brackenridge, Pa., No. 90-600.
8. Thin Films, Inc., Cambridge, Mass. Sodium filter: center wavelength, 5895 Å; peak transmission, 52 percent; 1-percent transmission bandwidth, 30 Å. Potassium filter: center wavelength, 7658 Å; peak transmission, 46 percent; 1-percent transmission bandwidth, 75 Å.
9. Philbrick Researches, Inc., Dedham, Mass. Type SP656.
10. A. P. I. Instrument Co., Chesterland, Ohio; mirror scale, 1-percent tracking accuracy.
11. We thank John Dirks and Maurice Abramow for assistance with the "tubule fluid" and the kidney-tissue extracts, and Judith Driver for help with the analyses.

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Brain Catecholamines: Relation to the Defense Reaction Evoked by Amygdaloid Stimulation in Cat

Abstract. *Electrical stimulation of the amygdala of cats, evoking a defense reaction, was associated with a reduction in the noradrenalin content of the brain and adrenal glands but not in the dopamine content of the brain. When stimulation resulted in quieting or sleep, catecholamine concentrations were unaffected. Changes in brain noradrenalin, therefore, appear related to production of the defense response and not to nonspecific amygdalofugal pathway excitation.*

In a previous study (*1*) we showed that the production of a defense reaction (*2*) or "sham rage" in the cat by electrical stimulation of the amygdaloid nucleus results in changes in catecholamines of the brain and adrenal glands. Both the noradrenalin (NA) content of the telencephalon, brainstem, and adrenals, and the adrenalin (A) content of the adrenals are significantly reduced while the dopamine (DA) content of the cerebrum is unaffected. It is not known, however, whether the chemical changes are specifically related to the induction of excited behavior by the brain stimulus or are the result of nonspecific activation of amygdalofugal pathways. In the study described here, we attempted to delineate the relationship between the chemical and behavioral consequences of amygdaloid stimulation. Changes in the NA and DA content of the brain and the NA and A content of the adrenals were compared in cats in which the stimulus produced rage and cats in which the stimulus resulted in quieting, sedation, or sleep.

The physiological and chemical methods are described in detail elsewhere (*1*, *3*). We studied 22 adult cats of both sexes; some had been included in our previous study (*1*). Three cats served as nonoperated controls. Under Nembutal anesthesia the other 19 animals had single stainless-steel Hess electrodes, insulated except for 2 mm at the tips, placed stereotaxically in the brain and fixed to the skull with dental cement. A screw in the calvarium was the indifferent (anodal) electrode. In 17 of the cats, electrodes were implanted in the right amygdaloid region. Six of these cats were not stimulated electrically and thus served as operated