forces near the base that no single measurement of threshold or subthreshold force is significant. Table 1 shows the means of six measurements with illumination and of 24 tests in darkness. From these evaluated means of forces for geotropic responses and no responses, one can only say that the threshold value lies somewhere between them, perhaps nearer to the mean for a distinct response. The tenfold difference between tests in darkness and in a lighted room agrees with previous observations (*2*) of greater sensitivity in darkness. Comparison with Czapek's estimate that an acceleration of 0.980 cm/sec^2 is threshold suggests that his failure to wait more than 8 hours for the effect of the force of 0.490 cm/sec^2 (compare our mean of 0.507) made his estimate double the true value for geotropic response in the light.

These decisive effects of very low centrifugal forces and the possibility of their production by slight vibrations must be considered if seedling plants are ever grown in a gravity-free state, as in manned space ships. The very low requirement of 0.044 cm/sec^2 for a mechanical force in darkness may also have a bearing on acceptable theories of graviperception (*3*).

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19 September 1960

Production of Polydipsia in Normal Rats by an Intermittent Food Schedule

Abstract. Marked polydipsia was produced in all animals trained to press a bar for food pellets on a 1-minute variable-interval schedule. It is suggested that since this feeding arrangement produces a sustained, high fluid intake in the normal, unrestrained animal, it might serve as a useful tool in the study of renal function.

Long-lasting, high rates of fluid ingestion can be roughly characterized as either metabolic or regulatory polydipsia. In the first case, high intake is a direct result of abnormal fluid losses (for example, as in diabetes insipidus), while regulatory or primary polydipsia usually is assumed to originate from a central nervous defect which stimulates neural thirst centers. Regulatory polydipsia can also be produced by chronic shifts in the volume and composition of water compartments of the body (for example, by sodium depletion).

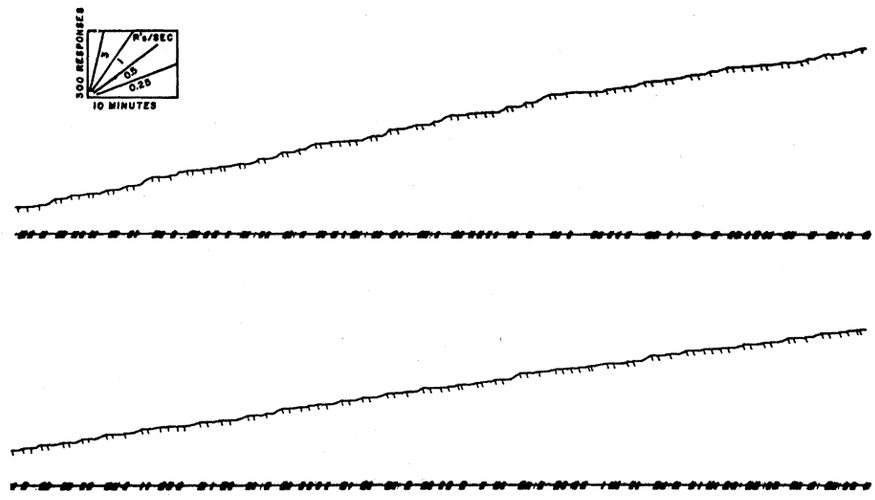

Fig. 1. Record of one complete VI-1′ session of bar-pressing for food pellets, showing a burst of drinking on the lower trace after delivery of the pellet (rat No. 6-3).

This report (*1*) concerns the production, in rats, of a type of regulatory polydipsia which, in human beings, would be classed as psychogenic.

Fourteen female, experimentally naive, albino rats (*2*) with a mean starting weight of 264 g were maintained at 70 to 80 percent of their starting weights on a 1-minute variable-interval (VI-1′) schedule of food reinforcement. Under this schedule, a bar-pressing response produces a 45-mg food pellet (*3*) at varying time intervals, the average of which is 1 minute (range, 3 seconds to 2 minutes). The variable interval (VI) schedule is designed so that no feature of the animal's behavior or the external stimulating conditions can inform the animal precisely when a bar-press will be reinforced by a food pellet. All presses occurring between the times when a pellet is readied for delivery by switching circuitry are ineffective but are recorded.

The animals were given daily experimental sessions lasting 3.17 hours. Water was available 24 hours per day. During the session a drinkometer recorded every lick from a glass drinkink spout attached to a calibrated reservoir. In the animal's home cage water was available from a Richter tube.

Figure 1 is the record of one complete session for rat No. 6-3. Bar-pressing moves the pen up (the moves are cumulative), and the delivery of each food pellet is indicated by a short vertical line. The lower trace records every 12th lick on the water spout. A characteristic behavior pattern is evident. Shortly after a pellet is earned a burst of licking ensues, followed by a return to bar-pressing until the next pellet is delivered. Post-pellet drinking is usually so prolonged that the pellets which are potentially available at the shorter intervals (for example, 3 seconds, 10 seconds) are not earned as rapidly as they might be. In fact, although the animals are deprived only of food, a high proportion of their session time is spent in drinking.

Table 1 gives the mean water intake for all animals (i) in their home cages prior to the start of the experiment, with food freely available; (ii) during the daily session; and (iii) in their home cages between sessions. The average value for intake during the sessions is 3.43 times higher than the mean pre-experimental, 24-hour value. This difference is all the more impressive if we note that the usual effect of food deprivation in the rat is a sharp decrease in water intake (*4*).

The polydipsic effect develops rapidly. It is usually quite evident in the first VI-1′ session and often fully developed by the second session. It has

Table 1. Mean water intake of rats not deprived of water, during the pre-experimental (free food) period; during the VI-1′ session of bar-pressing for food; and in the cage between sessions (no food).

Rat No.	Mean water intake (ml)		
	Pre-exptl. (24 hr)	Exptl. session (3.17 hr)	In cage (20.83 hr)
1-1	36.50	77.33	0.83
1-2	41.50	106.00	1.00
6-1	22.00	67.66	0.50
6-2	29.00	121.00	1.66
6-3	16.60	98.00	0.83
6-4	23.80	79.33	0.33
6-5	18.20	146.33	0.50
6-6	29.50	99.33	0.33
6-7	28.00	81.33	0.83
PO-1	*	71.00	2.50
PO-2	*	76.66	0.16
PO-3	*	89.50	0.66
PO-4	*	81.00	2.50
PO-6	24.50	100.66	0.66
Av.	26.96	92.51	0.95

* Not measured.

never failed to develop in an animal exposed to this schedule. Further experiments are in progress to elucidate the conditions necessary and sufficient for this effect.

The methods typically used to produce a sustained diuresis in the intact animal are loading the stomach with water or administering sodium chloride, mannitol, or other diuretic agents. Another method forces intake by making shock avoidance contingent upon licking (*5*). The arrangement described in this report results in voluntary drinking at a sustained high rate in the unrestrained, normal animal, without the various traumatic concomitants of the above methods.

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References and Notes

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10 October 1960

Selective Localization of Tetracycline in Mitochondria of Living Cells

Abstract. The property of tetracycline to induce fluorescence has been used to determine its localization in living cells. It was found that this antibiotic, as well as the related antibiotics oxytetracycline and chlorotetracycline, specifically combines with the mitochondria of living cells, either in tissue culture or in fresh preparations from various organs.

In the search for a fluorophor which would selectively localize and remain localized on mitochondria in living cells, a number of substances were tried with varying results. Benzpyrene and related carcinogens, as used by Graffi (*1*), localized on mitochondria and on fat droplets. Glycerol was employed as a vehicle. Using acridine orange, Wolf (*2*) found that the distribution and size of metachromatic granules in viable cells suggest that these granules may be mitochondria. With this florophor we found a variable fluorescence, which made observations difficult to interpret.

Our attention was drawn to the work of Milch *et al.* (*3*) and Rall *et al.* (*4*), who studied the gross distribution of the tetracycline group of antibotics in normal and neoplastic tissues. The possible use of antibiotics would have the added advantage over use of many fluorescent dyes in that antibiotics would not markedly interfere with the development of living cells, and would simultaneously supply further information regarding the mode of action of tetracyclines.

Milch *et al.* reported that bone fluorescence persisted for at least 10 weeks when a single, small parenteral dose (0.3 mg/kg) of tetracycline was administered to freshly frozen sections of several species of laboratory animals. This induced fluorescence disappeared from all tissues except bone within 6 hours after injection.

In the present study, observations on living cells are reported by means of phase-contrast and fluorescence microscopy.

Monkey kidney tissue-culture cells, grown in medium 199 with 2 percent calf serum and maintained in Eagle's basal medium on submersed coverslips, were exposed to 10 or 20 μg of tetracycline per milliliter of medium. (The amount of tetracycline currently used in the antibiotic mixture added to tissue culture media to suppress bacterial growth is about 10 μg/ml.)

Photomicrographs were made of the same cell, first by phase-contrast (Fig. 1), then by fluorescence microscopy (Fig. 2). The mitochondria showed intense fluorescence. Nuclei, vacuoles, interparticulate cytoplasm, and cell boundaries appeared dark. Some decrease in the intensity of the yellow fluorescence occurred within a few seconds after exposure to ultraviolet radiation. In similar preparations made of the same material, and in the continued presence of the antibiotic, the cells were alive after 4 days and the fluorescence was still localized in or on the mitochondria. At concentrations of 100 μg or more cell damage occurred (*5*). When the tetracycline-treated material was resuspended in tetracycline-free medium, the fluorescence decreased gradually over several hours until the mitochondria were only faintly fluorescent.

No fluorescence was observed in chromosomes in dividing cells which had been previously treated with the antibiotic. Strain L cells showing a centrosome area did not show fluorescence of the centrosome. Similar results were obtained with tissue cultures which were exposed to oxytetracycline or chlorotetracycline (10 to 20 μg/ml of medium).

To test fresh isolates, adult mice were injected intraperitoneally with 1.0 ml of saline containing 2000 μg of tetracycline per milliliter, or approximately 100 μg/g of mouse; after 2 hours the animal was killed, and brain, liver, and spleen fragments were collected in 0.88*M* sucrose. Fresh microscopic preparations of these organs were made. The tetracycline was again localized on the mitochondria. Liver mitochondria

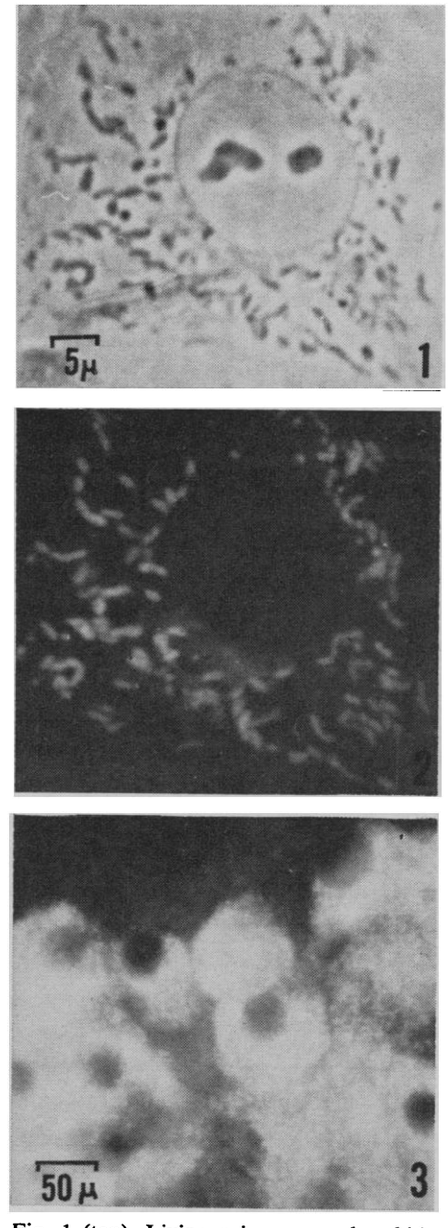

Fig. 1 (top). Living primary monkey kidney cells after a 3-hour exposure to 20 μg of tetracycline per milliliter (Chas. Pfizer). Phase contrast. Fig. 2 (middle). Same field as Fig. 1, showing mitochondrial fluorescence. Zeiss W microscope with mercury HBO-200 light source, used with two BG-12 exciter filters and barrier filter OG-5. Fig. 3 (bottom). Mouse liver tissue fragment mounted in 30 percent sucrose. Nuclei appear black in contrast to strong fluorescence by mitochondria. The animal was injected intraperitoneally with 2000 μg of tetracycline prior to death and observation.