an additional 1% of the absorption placed on the column.

The yield of each fraction in terms of the ultraviolet absorption at 260 mμ initially placed on the column is summarized in Table 1. Of the initial absorption 95.7% was recovered.

TABLE 1

Fraction	Initial Absorption at 260 mμ (%)
Bases and nucleosides	21.6
Desoxycytidylic acids	10.8
Thymidylic acid	10.2
Desoxyadenylic acid	21.5
Desoxyguanylic acid	14.8
Eluted with 2 *M* buffer	15.8
Eluted with 1 *N* HCl	1.0
Total recovery	95.7

Ion exchange analyses of the digest resulting from the action of the pancreatic enzyme alone (for 80 hr at 37° C) have consistently revealed that only very small amounts of the four mononucleotides are released. Individually each accounts for less than 1% of the total initial ultraviolet absorption; over 95% of the absorption remains in the form of polynucleotides. This result is in agreement with the conclusions of Zamenhof and Chargaff (*6*).

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An Electronic Drinkometer

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The need for an apparatus for continuously recording the fluid intake of animals under a variety of experimental conditions has long been obvious in the study of thirst. Such a recorder is no less important, however, in other cases where fluid intake is measured, such as in studies of alcohol consumption or specific hungers for solutions of vitamins, salts, sugars, etc. The problem is to measure not only how much of a given fluid an animal drinks, but also when and how fast it drinks.

Various methods have been used in the past to record the course of drinking. The most obvious but most time-consuming is to take periodic readings of the fall of fluid in a graduated container. Another is to train the animal to operate a mechanism such as a lever-box in order to obtain measured amounts of fluid (*1*). A graph of the number of operations or number of lever pushes gives a complete record. But this method makes the animal work for its fluid and also rations the fluid in arbitrary amounts per response. A third method is the float-kymograph technique (*2*). The fall of fluid in a container is continuously recorded on a kymograph by a stylus that floats on the surface. The float-kymograph is a very cumbersome apparatus, and is also rather insensitive, since a sizable amount of fluid must be withdrawn from the system before the stylus is displaced.

The electronic drinkometer was designed to get around most of these shortcomings. The principle is simple. Whenever the animal touches the fluid it is to drink, it completes an electronic circuit that activates the pens of a kymograph. In the apparatus particularly designed for the rat, the main circuit consists of a 9-v battery source, a 20-megohm resistance, and a 6SN7 vacuum tube. One lead from this circuit goes to the wire-mesh floor of the rat's cage; the other makes contact with the fluid contained in an inverted graduate, fitted with a glass nipple from which the animal drinks. Every time the rat comes in contact with the fluid, it completes the circuit and biases the grid of the vacuum tube. Each change in grid bias fires a No. 850 advance relay, which closes another circuit to the magnets of a signal marker. Eight complete recording units of this sort were used to mark the waxed tape of a kymograph and thus provide permanent records of drinking.

In order to obtain the most sensitive records, the nipple of each graduate was brought up to a small opening in the wire-mesh wall of each cage so that only the rat's tongue could touch the fluid. As the rat drank, each tongue lap produced a single mark on the kymograph tape. With the tape running at 2.0 mm/sec, individual tongue laps could be counted, and the drinkometer could be calibrated for amount of fluid per tongue lap. At the slower tape speed of 0.5 mm/sec, used for prolonged recording, individual tongue laps could not be distinguished, and calibration was done in terms of the amount of fluid taken per millimeter of marked tape.

The actual process of calibrating was greatly facilitated by the fact that the rat's tongue always laps water at a constant rate of 6 or 7 laps/sec. This rate is maintained under a wide variety of conditions. For example, the rate is the same in the last 5 min of drinking in a 2-hr period as it is in the first 5 min. Furthermore, the rate is constant whether the rat has been deprived of water 6 hr or for as long as 7 days.

With the rate of lapping constant, it is possible to compute the total number of tongue laps made in a 2-hr period by measuring the amount of tape marked. The amount of water taken per tongue lap can then be calculated from the total water intake for that period, recorded to the nearest .5 ml from the graduated water bottles. The rats in this study averaged between .004 and .005 ml water/tongue lap. Essentially the same values were obtained when the rats were allowed to drink 1 or 2 ml water.

Variability in the amount of water taken per tongue lap was kept at a minimum in this work by holding the following factors as constant as possible: the size

of the rats, the size of the nipple openings, and the amount the animals had to stretch their tongues to reach the water. Male rats, about 5 months old, weighing around 350 g, were used. Nipple openings varied only between 3.6 and 3.9 mm, and each rat always drank from the same nipple. Finally, the nipples were always brought up to a fixed position at the cages so that tongue stretching was constant. As an added precaution to insure against wide rat-to-rat and condition-to-condition variations, the amount of water taken per tongue lap was always computed individually for every rat and every condition of drinking.

For practical purposes, measurement of drinking down to the last tongue lap is not necessary, of course. In fact, it is quite satisfactory in most cases to calibrate the drinkometer simply in terms of the amount of water ingested per millimeter of fully marked tape. With the kymograph going at 2.0 mm/sec, it can be shown that rats always drink at a rate of about 0.03 ml/sec. Even second-by-second records of drinking are too detailed for most purposes. It is often sufficient to determine the amount of drinking in each second and then make accurate minute-by-minute plots of the course of drinking (Fig. 1).

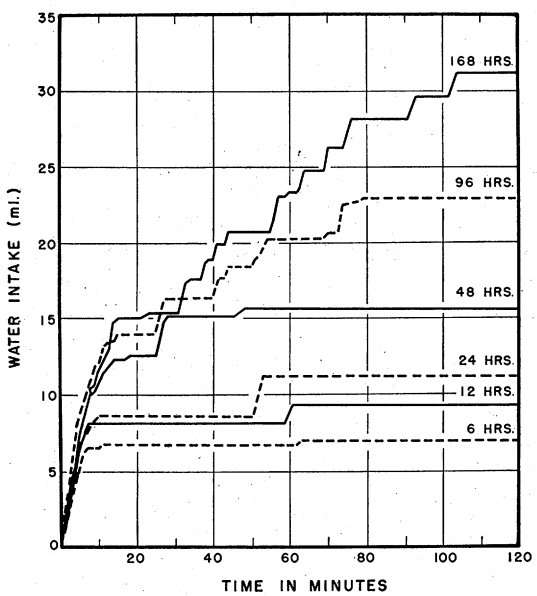

FIG. 1. Minute-by-minute plot of the rate of drinking of one rat following different amounts of water deprivation.

As Fig. 1 illustrates, the drinkometer yields detailed data which are very valuable in analyzing drinking behavior. It is clear that after water deprivation, the rat will drink virtually without interruption for as long as 8 min. Then it will rest and drink by turns. The more severe the deprivation, the less time the rat will spend resting and the longer it will drink each time it returns to the water bottle. Since drinking is always at a constant rate, the longer the water deprivation a rat has suffered, the more it will drink in a 2-hr period.

The drinkometer has also been used to trace the course of drinking over longer periods of time, up to 48 hr. In this case the kymograph tape was run at 0.5 mm/sec, and excellent records were obtained showing when the rat drinks and how much time it spends drinking during a normal day. The typical rat in our study does 78% of its drinking in the dark and devotes only about 20 min in every 24 hr satisfying its water needs.

The foregoing results illustrate only a few of the possible uses of the drinkometer. Nevertheless, they are sufficient to make several important features apparent. First, the drinkometer is simple, inexpensive, economical of space, and easy to operate. Second, it provides an extremely sensitive, continuous record of all the drinking an animal does over any reasonable period of time. Third, it will record fluid intake in any normal, free-drinking situation without disturbing the animal, making it work, or requiring that it be trained. Finally, although the drinkometer was designed for the rat, it could be used equally well with any mammal and probably any bird or reptile.

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The Role of Thiouracil in the Induction, Growth, and Transplantability of Mouse Thyroid Tumors

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Goitrogenic drugs, such as thiouracil, are believed to act directly on the thyroid gland to block reactions essential for the synthesis of thyroid hormone. The resulting diminished level of the thyroid hormone in the circulation is thought to provoke an increased output of thyrotrophic hormone (TSH) by the anterior lobe of the hypophysis, which acts on the secretory epithelium of the thyroid, producing cellular hypertrophy and hyperplasia. Thyroid glands of thiouracil-treated mice weighing 3–30 times more than those of control mice have been produced in this laboratory, but in spite of extreme hypertrophy involving both cellular hypertrophy and hyperplasia, no evidence of neoplasia was found in such thyroid glands (*1*). Nodules in the lungs, believed to represent true metastases from thyroid glands, were found, but such nodules did not display any more evidence of neoplasia than did the thyroid of the animal in which they occurred.

It was suggested by one of us (CDG) that prolonging the period of hyperplasia beyond the lifetime of any one mouse by transplanting the hyperplastic thyroid to other mice might result in further alteration, eventual neoplasia, and possibly even malignancy. The test was carried out by culturing thyroid tissue *in vivo* in a homologous growth medium where simultaneous