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).

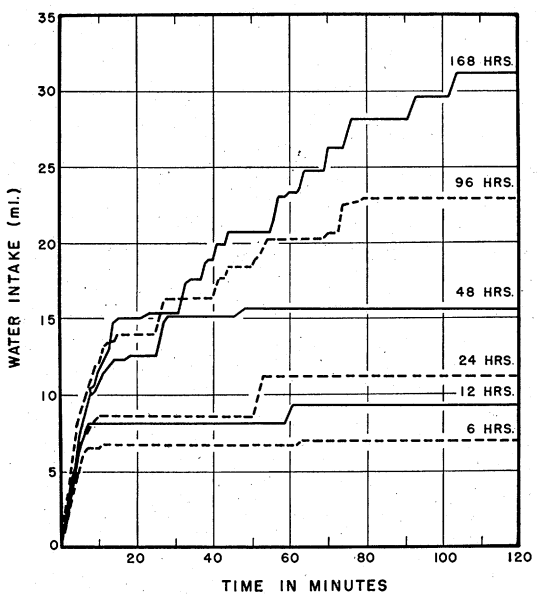

FIG. 1. Minute-by-minute plot of the rate of drinking of one rat following different amounts of water deprivation.

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

TABLE 1

ORIGIN AND TREATMENT OF THYROID TISSUES BEFORE AND AFTER TRANSPLANTATION

Expt. No.	Date original host started on thiouracil	Months on thiouracil before implantation	Date of original implantation	Original tissue implanted	Diet of host before implantation	Maximum No. implant generations*	Maximum time of thiouracil ingestion† (months)	Earliest appearance of complete autonomy‡ generation
177	9/14/44	16	11/30/45	Thyroid	145§	10	74	0
180	9/14/44	18	3/8/46	"	145	16	74	9
183	11/14/45	16	3/14/47	"	157‖	11	62	3
199	8/6/46	20	3/23/48	Lung metastatic nodule	160¶	4	51	0

* Transfer of original tissue to a new host represents beginning of the first implant generation. Numbers in this column refer to number of transplantations made subsequent to the original starting tissue.

† Includes time in original host plus time in transplant host.

‡ Complete growth autonomy means ability of tissues to continue growth without additional TSH stimulation, and began in the transplant generation indicated by the figures in this column.

§ Contained 0.5% thiouracil.

‖ 2-Acetylaminofluorene added at a level of 0.05% to the stock diet containing 0.5% thiouracil = diet 157.

¶ Diet 160 contained 0.375% thiouracil.

and continuous TSH stimulation was maintained indefinitely through ingestion of thiouracil. Small pieces of hyperplastic thyroid tissue from mice who had ingested thiouracil for many months (Table 1) were transplanted to young mice also ingesting thiouracil by means of sterile trocar. Every 3–6 months, or as soon as the implant had increased sufficiently in size, the tissue was retransplanted. The transplanted tissue developed an extensive vascular bed very rapidly and grew well. After a few transplantations, and when sufficient tissue became available, it was also implanted into mice receiving the stock diet devoid of the goitrogen. Thus it was possible to test frequently the growth potentialities or autonomy of the tissue in normal animals.

Three lines of thyroid tissue implants that have been produced by the above procedure are illustrated by experiments 177, 180, and 183 in Table 1. These implant lines originated from the thyroids of mice that had been fed thiouracil[1] for 16–18 months. Hyperplastic thyroid-cell growths and tumors, direct descendants of the original thyroids, have now been transferred through 10–16 generations over a period of 4–5 years (Table 1). Transplant line 183 is further complicated, however, because it originated from a mouse that also received .05% 2-acetylaminofluorene (AAF) in the thiouracil-containing diet. Although complete autonomy was first observed in this line with the third implant generation, and not until generation 9 in line 180, the possible relation of AAF thereto must await further study. A fourth thyroid tumor line, 199, originated from a thyroid metastasis transplanted from the lungs of a mouse after ingestion of thiouracil for 20 months (Table 1). This line has now been carried through 4 transplant generations, with growth potentialities similar to those from hyperplastic thyroids, even though it has not yet attained complete growth autonomy.

Several sublines have developed complete autonomy because increased TSH stimulation, induced by thiouracil ingestion, is no longer required for continued growth. Thyroid tumors often exceed 2 cm in diameter in 2–6 months, grow either intramuscularly or subcutaneously, and eventually kill the host. Metastases also occur in the lungs. One autonomous subline has been transferred to control mice for 10 consecutive generations. The histologic structure of the transplants of this tumor subline carried in hosts receiving thiouracil was in general similar to that of the transplants carried in hosts fed the control diet (*2*). Other sublines still require TSH stimulation, and when such stimulation is withdrawn, by removing thiouracil from the diet of the host, the implant undergoes regression.

Recently Hall and Bielschowsky (*3*) reported morphological evidence that malignancy also appeared in the thyroid of rats receiving a goitrogen for 1½ years. These investigators concluded that the essential factor in the pathogenesis of the experimental tumors of the rat thyroid was the continuous increased stimulation by TSH. Money and Rawson (*4, 5*) consider that, in addition to the over-stimulation of the thyroid by TSH, the iodine deficiency produced through the action of antithyroid drugs may also be involved in the process of thyroid tumor formation in rats.

Bielschowsky *et al.* (*6*) have transplanted the experimentally produced thyroid tumors of rats only when a state of thyroid deficiency was maintained with its consequent increased output of TSH, which shows that the tumors were not autonomous, but could be maintained only under the same conditions responsible for the production of the original tumor.

The work reported here is believed to furnish the first experimental evidence that autonomous thyroid tumors can be produced in mice after prolonged ingestion of thiouracil and presumably similarly pro-

[1] The thiouracil used in these experiments was generously supplied by Lederle Laboratories, Pearl River, N. Y., through the courtesy of S. M. Harding.

longed TSH stimulation. The fact that the mouse thyroid tissues were continued for a longer period in a continuous state of stimulation than were those of the rat may be one explanation for the successful development of thyroid tumors in mice transplantable to normal animals. Wollman, Morris, and Green (*7*), in preliminary studies of the functional activity of 4 tumor sublines, found that 4 transplantable tumors showed a wide variation in ability to concentrate inorganic iodide or to convert it into thyroxine. These studies are suggestive of the potential value of experimental thyroid tumors as an aid to a better understanding of thyroid physiology and cancer. The peculiarities of several sublines of thyroid implants and autonomous tumors in relation to their functional, histological, and cytological characteristics will be reported in detail elsewhere.

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Comments and Communications

Radioactivity of the Hot Springs of Tiberias[1]

SEVERAL experiments that have been carried out in the past (M. Buchmann, *J. Palestine Med. Assoc.*, **25**, [8], 4 [1943]) appeared to indicate that the thermal springs located at Tiberias, Israel, are radioactive. In view of the fact that remarkable curative properties have been ascribed to these springs since antiquity, a new investigation, using the improved techniques that have become available during the past decade, has been initiated.

The springs issue at a temperature of 63° C. Their chief constituents, by weight, are the dissolved chlorides of sodium, calcium, and magnesium. Kodak N.T.A. plates with an emulsion thickness of 25 μ have been exposed by immersion in samples of water from the springs, in samples that were evaporated down to various increased concentrations, and in solutions of salts crystallized from the springs. Exposures were also effected by several other methods, including suspension of plates above the springs and evaporating samples on the surface of the plates. The age of samples varied from a few hours to several years.

Some of the exposures resulted in the appearance of peculiar structures. The dimensions and number per unit area of the phenomena were such that they could reasonably be attributed to α-radiation, but their spurious nature became evident upon close examination. Consequently, in the case of the relatively few exposures in which these pseudo tracks and stars appeared, they were excluded from the α-count.

Counts of α-tracks and stars failed to indicate any consistent activity above background. Control was maintained by addition of minute amounts of very weak standard solutions of radium barium bromide to samples and noting the increased α-counts. These control experiments indicate that samples emitting α-radiation energetic enough to produce discernible tracks—i.e., $E > 1.7$ mev, and of an activity exceeding 10^{-13} curies/cm^3—would have yielded statistically significant net counts. β-radiation, as well as short half-life, or very low-energy α-activity would have escaped detection in these experiments.

Further research, making use of several types of counters, is being carried out in an effort to check on these possibilities, as well as to revise the present upper limit to the α-activity.

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Climate and Culture—New Evidence

IN 1932 (*1*) I suggested that the remarkable flowering of the mound-building cultures in the upper Mississippi and Ohio drainage had been coincident with a prolonged warm-dry phase of climate to which the name xerothermic has been applied. This period had been marked by a shift of continental vegetation, including grasslands, toward the Northeast. Such a movement was deduced by Gleason (*2*) on the basis of plant distribution, and later amply confirmed by pollen analysis of fresh-water sediments.

I also suggested that the replacement of the mound cultures by less advanced forest cultures was related to the subsequent cooling and increase of humidity. These conditions favored the return of forests.

Since 1948, with the active encouragement of Mexican colleagues and financial assistance from the Geological Society of America and the Wenner-Gren Foundation (Viking Fund), I have had an opportunity to study pollen sequences in relation to archaeology within the Mexico City Basin. This study has established the fact that the transition Late Archaic–Teotihuacan I took place during a prolonged dry period whose position in the sequence corresponds to our xerothermic.

The recent carbon 14 dating by Arnold and Libby (*3*) assigns this transition and the Hopewell mound