4% solution used in the human studies was tested by injecting 0.5 g/kg into mice. The solution used was between 6 and 12 months of age. None of the 10 animals given the dye showed any evidence of toxicity.

When one compares the usual human dose, 5 ml 4% (0.2 g), with the LD_{50} for mice (2.26 g/kg), it is evident that there is a large safety factor involved even if the dye should inadvertently be injected intravenously. Furthermore, when one considers that most of the stained area is removed during surgery, it is probable that the total amount of residual dye is of no practical importance. Applying the toxicity criteria of Hodge and Steiner (*6*) reveals that Pontamine Sky Blue would be classed as a slightly toxic material.

A full report of the clinical utility and duration of skin staining caused by Pontamine Sky Blue will be reported in detail elsewhere.

References

1. BRAITHWAITE, L. R. *Brit. J. Surg.*, **11**, 7 (1923).
2. MCMASTERS, P. E., and PARSONS, R. J. *J. Exptl. Med.*, **69**, 247 (1939).
3. HUDACK, S. S., and MCMASTERS, P. D. *Ibid.*, **57**, 751 (1933).
4. WEINBERG, J., and GREANEY, E. M. *Surg. Gynecol. Obstet.*, **90**, 561 (1950).
5. LITCHFIELD, J. T., JR., and WILCOXON, F. *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).
6. HODGE, H. C., and STEINER, J. *Am. Ind. Hyg. Assoc. Quart.*, **10**, 93 (1949).

Ion Exchange Separation of Desoxyribonucleotides[1]

Robert L. Sinsheimer and James F. Koerner

Department of Physics, Iowa State College, Ames

As has been implied by Cohn (*1*), the ion exchange methods developed by him for the separation of ribonucleotides (*2*) may be readily adapted to the separation of desoxyribonucleotides. Since no procedure for this separation has been published, it would seem worth while to outline the technique developed in our laboratory.[2]

The mixed desoxyribonucleotides were prepared by a two-stage enzymatic hydrolysis of highly polymerized desoxyribonucleic acid (DRNA). Two hundred mg of DRNA, prepared from calf thymus according to the method of Mirsky and Pollister (*3*), was dissolved in 20 ml 0.5 *M* acetate buffer, pH 6.5. To this were added 10 ml 0.1 *M* $MgSO_4$ and 10 ml of a distilled water solution of crystalline pancreatic desoxyribonuclease (Worthington Biochemical), containing 400 μg/ml. The enzymatic digestion was carried out for 72 hr at 37° C, under a layer of spectroscopically purified hexane, with constant stirring by magnetic stirrer.

The second stage of enzymatic digestion was carried out according to the method of Klein (*4*), using a nuclease preparation from intestinal mucosa, in the presence of arsenate to inhibit alkaline phosphatase also present in the preparation. The pH of the digest resulting from the action of the pancreatic enzyme was adjusted to 8.5 with 2 *N* NaOH. To the digest were added 10 ml 0.36 *M* $Na_3(AsO_4)_2$, 10 ml 1 *M* $NH_4OH \cdot (NH_4)_2SO_4$ buffer, pH 8.5, and 10 ml of a solution containing 17 mg/ml of the intestinal enzyme, prepared according to Klein, as modified by Brady (*5*). Digestion was carried out for 20 hr at 37° C under hexane, with constant stirring.

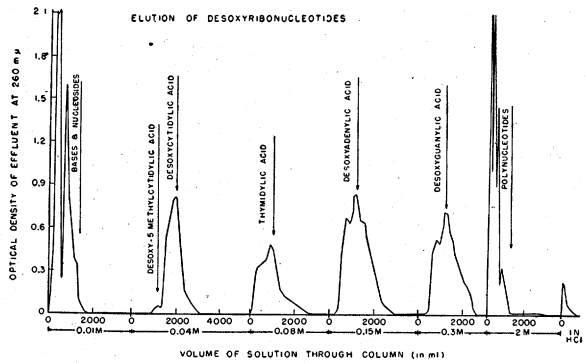

FIG. 1.

The pH of the digest was adjusted to 4.7 with glacial acetic acid to precipitate the intestinal preparation, which was then centrifuged out. The centrifugate was washed with 0.01 *M* acetate buffer, pH 4.7, and the washings were added to the previous supernatant. The pH of the combined solutions was adjusted to 9.0, and the volume made up to 250 ml.

This solution, containing mixed bases, nucleosides, nucleotides, and some undigested polynucleotides, was then added to the ion exchange column, π cm^2 × 11 cm, Dowex A-1 resin, 250–500 mesh[3] in the acetate form. Because of the marked lability of the purine desoxyribonucleotides to acid, it is desirable to employ procedures that avoid any prolonged contact with solutions of low pH. For this reason, elution from the ion exchange column was carried out in buffered solution at pH 4.3.

The elution procedure is indicated in Fig. 1. Bases and nucleosides, which accounted for 21.6% of the ultraviolet absorption of the material added to the column, were eluted with 0.01 *M* acetate buffer, pH 4.3. Desoxy-5 methyl cytidylic acid and desoxycytidylic acid were eluted, with incomplete separation, with 0.04 *M* buffer. If it is desired to separate these completely, they may be recycled on an acetate column, using buffer at pH 4.7 for elution (*1*). Thymidylic acid was removed with 0.08 *M* buffer, desoxyadenylic with 0.15 *M* buffer, and desoxyguanylic with 0.3 *M* buffer.

Subsequent passage of 2 *M* acetate buffer, pH 4.3, through the column removed a number (at least four) of different substances with varying absorption curves, presumably polynucleotides. These are being further investigated. Finally, treatment with 1 *N* HCl removed

[1] This investigation was supported in large part by a grant from the Rockefeller Foundation.

[2] Since submission of this paper, analogous procedures have been described by Hurst *et al.* (*J. Biol. Chem.*, **188**, 705 [1951]) and by Volkin *et al.* (*J. Am. Chem. Soc.*, **73**, 1533 [1951]).

[3] This resin was obtained through the courtesy of L. Matheson, of the Dow Chemical Company.

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*).

References

1. COHN, W. E. *J. Am. Chem. Soc.*, **72**, 2811 (1950).
2. *Ibid.*, 1471.
3. MIRSKY, A. E., and POLLISTER, A. W. *J. Gen. Physiol.*, **30**, 117 (1946).
4. KLEIN, W. In Bamann and Myrbäck (Eds.), *Methoden der Ferment-Forschung*. New York: Academic Press, 1924–41 (1945).
5. BRADY, T. A. *Biochem. J.*, **35**, 855 (1941).
6. ZAMENHOF, S., and CHARGAFF, E. *J. Biol. Chem.*, **187**, 1 (1950).

An Electronic Drinkometer

J. Harry Hill and Eliot Stellar

Department of Psychology,
The Johns Hopkins University, Baltimore, Maryland

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