now been demonstrated in marmosets indicates that this animal may be suitable as a subject for further investigation along these lines.

Two members of the Callithricidae were studied earlier (*16, 17*), the silky marmoset *(Callithrix chrysoleucos)* and the red-mantled temarin [*Tamarinus* (or *Leontocebus*) *illigeri*]. Members of *Callithrix* and of *Tamarinus* were found to have a complement of 46 chromosomes; this finding is in accord with the finding for the genera (*C. jacchus* and *L. rosalia)* investigated in our study. While the X chromosome is a large metacentric element in all four of these genera of marmosets, differences exist with respect to the Y chromosome. The Y chromosome of the two genera studied by Bender and Mettler (*16*) was the smallest element and metacentric in structure. The best karyotypes of our *Callithrix jacchus* (Fig. 1) indicate that the Y chromosome of this genus is a minute acrocentric element. Most commonly, however, the preparations show only a small speck of chromatin material; the Y is distinctly the smallest member. In *Leontocebus rosalia* the Y chromosome, by contrast, is metacentric and cannot be clearly distinguished from the smallest pair of autosomes (Fig. 2). Structural differences in the autosomes in the genera of our study can also be seen in Figs. 1 and 2, but these cannot be compared with the autosomes of the animals that were reported by Bender and Mettler (*16*), in the absence of photographic representation of the karyotypes of their test animals.

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## Ribonucleotides of RNA: Separation by Chromatography on Sheets of Diethylaminoethylcellulose

*Abstract.* The four main ribonucleotides of RNA can be separated on diethylaminoethylcellulose paper sheets, with results comparable to column chromatography, by irrigation with 0.05*M* formic acid for 2 to 3 hours, and then reversing the direction of irrigation with 4*M* formic acid.

No single developing system has been reported for the successful separation of the four main mononucleotides which are obtained from an alkaline digest of RNA. The migratory behavior of various ribonucleoside mono-, di-, and triphosphates on an ion-exchange cellulose was reported by Randerath, who used several developing systems (*1*). In addition, the migration of various oligonucleotides was explored by Bollum (*2*). Smillie developed a procedure for separating the ribonucleotides of adenylic, uridylic, guanylic, and cytidylic acids (AMP, UMP, GMP, and CMP, respectively) by using a secondary migration in a direction opposite to the first one (*3*). The use of paper impregnated with ion-exchange resin precludes the possibility of observing the ultraviolet-absorbing compounds directly (*3*). For this reason, the use of diethylaminoethylcellulose paper is more desirable, and the present report describes its use for successful separation of the above mononucleotides from one another and from such contaminants as orthophosphate and ribonucleosides.

Sheets of DEAE-cellulose (18 by 22 inches, Whatman DE-20) were suspended in chromatography tanks and supported by Kurtz-Miramon glass frames for descending chromatography (*4*). Commercially available reagent-grade formic acid (97 to 98 percent) was used to prepare the developing solutions.

The DEAE-cellulose sheets were converted to the formate form by irrigating, on a frame, with 1*M* formic acid for an interval that was two to three times that required for the front of the solvent to travel the length of the paper. The papers were then irrigated with water until the formic acid on the paper was less than 0.003*M*, as detected by taste. The papers were dried at 25°C. The migration of aqueous solutions on papers washed with formic acid was three times faster than the migration on sheets of the untreated Whatman DE-20; about 2½ hours were required for the front of aqueous solutions to advance the length (22 inches) of the washed paper. The positions of the nucleotides appeared as dark areas when the paper was illuminated with ultraviolet light of short wavelength (*5*).

Samples of ribonucleotides from the hydrolysis of RNA with KOH could be satisfactorily applied to the paper in amounts up to 0.1 ml per inch at the origin if they were neutralized with $HClO_4$ and the salt had been removed by cooling to 0°C. For analysis of larger samples of hydrolyzate, the nucleotides were absorbed on acid-washed Norit and subsequently eluted with 10 percent aqueous pyridine (*6*). This step also removed much orthophosphate. The capacity of the paper was such that 0.1 mg of each of the four ribonucleotides could be separated from one another.

Samples were applied so that the origin was 9 inches from the upper end of the paper. Irrigation with 0.05*M* formic acid in a descending manner was continued until the cytidylic acid had moved about 8 to 9 inches; at this

Table 1. Results of three paper chromatograms and one column chromatogram of ribonucleotides. RNA was hydrolyzed by 0.1*M* KOH, 18 hours at 37°C, acidified with $HClO_4$, and mixed with acid-washed Norit. The charcoal was washed with water and extracted with 10 percent aqueous pyridine. The pyridine was removed by drying. Samples were dissolved in 0.01*M* $NH_4OH$ and applied either to column or paper. Column chromatography on Dowex-1 was done according to the method of Cohn and Bollum (*7*).

| Ribonucleotide | Total counts recovered (%) | | | |
|---|---|---|---|---|
| | Paper | | | Column |
| CMP | 27.6, | 27.5, | 27.4 | 28.4 |
| AMP | 21.4, | 21.8, | 20.8 | 21.4 |
| GMP | 27.4, | 26.6, | 28.0 | 27.9 |
| UMP | 23.6, | 23.0, | 22.5 | 22.6 |

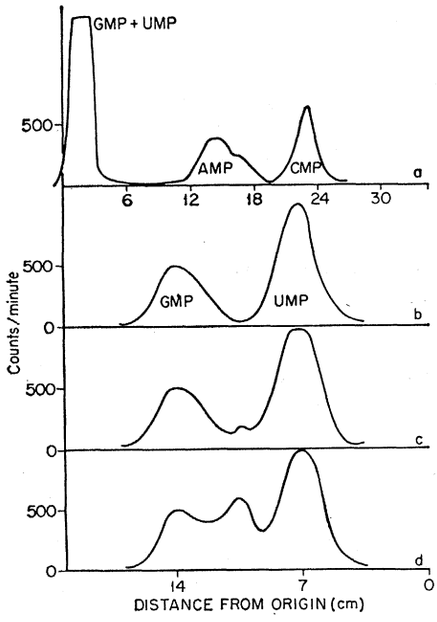


Fig. 1. Detection of contamination by radioactive $P^{32}$-orthophosphate. To each strip of DEAE-cellulose, formate form, charcoal eluate (3300 counts per minute) of alkaline hydrolysate of $P^{32}$-RNA was added. The separation of CMP and AMP after irrigation with 0.05*M* formic acid is shown by *a*. The paper was then cut at about 7 cm from the origin and irrigated in opposite direction with 4*M* formic acid; *b* shows GMP and UMP separation; *c* and *d* indicate the same separation in the presence of 140 and 660 counts per minute of $P^{32}$-orthophosphate, respectively.

time, the adenylic acid had moved about 4 to 6 inches, and uridylic and guanylic acids about 1 inch. The paper was dried at 25°C and was cut midway between the zones of adenylic acid and of the still unseparated guanylic and uridylic acids. The cut end of the upper piece was clamped on the Kurtz-Miramon frame and the paper was irrigated in the reverse direction with 4*M* formic acid. Guanylic acid migrated about twice as fast as uridylic acid and the former fluoresced under ultraviolet light when the paper was wet with 4*M* formic acid. The chromatograms could be removed from the tank for 2 to 3 minutes for observation without detriment to the separation of the nucleotides. The time for the first separation in 0.05*M* formic acid was about 2½ hours, and for the second about 45 minutes. Each ribonucleotide was eluted after chromatography with 1*M* ammonium bicarbonate. Because the latter sublimes readily, the nucleotides were thus free of extraneous salt (*7*). Radioactivity was measured by means of a Forro gas-flow Geiger tube with a 0.8 mg/cm² window for paper strips and a Tracerlab gas-flow Geiger counter with a 0.9 mg/cm² window for planchets.

Shown in Fig. 1 is the separation of ribonucleotides from the alkaline hydrolysis of RNA labeled with $P^{32}$-orthophosphate (*8*). Contaminating radioactive orthophosphate was readily detected since it migrates to a position intermediate between uridylic and guanylic acids (Fig. 1, *c* and *d*). In the absence of such contamination, the radioactive zones correspond exactly with the ultraviolet-absorbing zones. The chromatography was usually performed on strips of DEAE-cellulose about 1 inch wide so that the zones could not be wider than the window of the Geiger tube of the strip counter; narrower strips are too easily torn when wet.

Radioactive ribonucleosides were detected after chromatography in 0.05*M* formic acid since all four migrate more rapidly than cytidylic acid.

The quantitative distribution of radioactive phosphorus among the nucleotides which had been separated by DEAE-cellulose paper was determined and compared with that on a chromatogram prepared from a column of Dowex-1 according to the method of Cohn and Bollum (*7*). Table 1 shows that results with the paper method are comparable to those from the ion-exchange column.

This procedure would probably be adequate also for the determination of base ratios as measured spectrophotometrically, if the DE-20 papers were carefully cleaned for the purpose. For determining such ratios based on radioactivity, the method is satisfactory and convenient without modifications.

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## Force of Response during Ratio Reinforcement

*Abstract.* Sharp decline in response force after reinforcement and progressive force elevation over a sequence of unreinforced responses were observed for subjects in a fixed ratio lever-press situation. It is suggested that these systematic variations in force level may provide, through feedback, discriminable cues for behavioral regulation.

When a simple motor response is maintained with a schedule of intermittent food reinforcement, a characteristic and stable pattern of response frequency typically develops (*1*). Fixed-ratio (FR) is a major class of such reinforcement schedules, involving the reinforcement of every *N*th response.

Recent research (*2*) has shown that properties of behavior such as the peak force of response emission will be greater in magnitude and more variable during extinction than during continuous reinforcement. Since fixed-ratio schedules combine the experimental operations of reinforcement and extinction, peak force of response during reinforcement of this type was examined in order to determine if systematic changes in this response property occurred during the schedule performance.

The procedure involved a modification of the conventional fixed-ratio programming, which typically has single reinforced responses separated by sequences of fixed numbers of unreinforced responses. In order to observe progressive behavioral changes that might occur during reinforcement, sequences of several reinforced as well as unreinforced responses were used. By the convention of specifying the number of reinforced responses with a roman numeral, and the number unreinforced with an arabic numeral, a fixed-ratio schedule involving cycles of four successively reinforced responses and 12 successively unreinforced responses is designated FR(IV)-12. Within the respective sequences each cycle position is identified by the appropriate numeral.

The subjects were male albino rats of the Wistar strain, approximately 110 days of age at the beginning of experimentation. The animals were maintained under a food deprivation regimen, and food pellet reinforcement for a lever-press response was employed (*2*). A peak force of at least 2.5 g was required for all responses. Continuous reinforcement (approximately 250 re-