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 $0.05M$ formic acid is shown by a. The paper was then cut at about 7 cm from the origin and irrigated in opposite direction with $4M$ 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 $4M$ 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 $4M$ 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 $0.05M$ formic acid was about 2½ hours, and for the second about 45 minutes. Each ribonucleotide was eluted after chromatography with $1M$ 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 $0.05M$ 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 Nth 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-
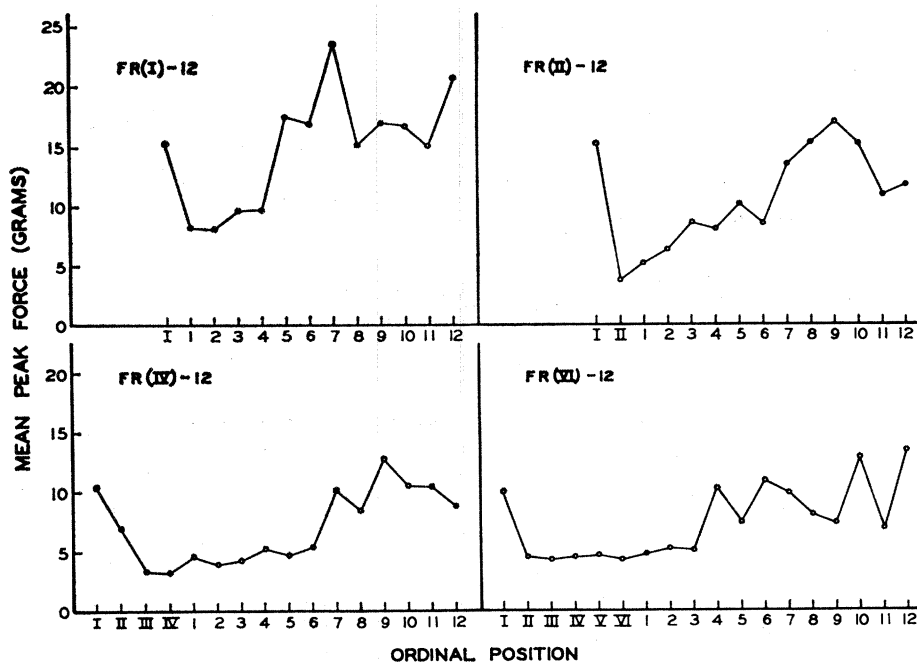
Fig. 1. The mean peak force of response during modified fixed ratio performance. The roman numerals indicate reinforced, and the arabic indicate unreinforced, ordinal positions of the cycle.

sponses) was conducted prior to fixed-ratio training.

Figure 1 shows the mean peak force of response at each ordinal position of the fixed-ratio cycles for four different animals, each under a different schedule. Each of the schedules required 12 unreinforced responses within a cycle. The number of reinforced responses varied with the subjects, as indicated in Fig. 1. The means are based on 20 to 25 cycles (20 to 25 responses for each ordinal position). The data represent the 12th consecutive day's performance on the particular schedule.

All subjects, regardless of the number of reinforced responses, show the same trend: The first reinforcement (position I) is followed by a sharp drop in the mean peak force of response. So long as reinforcement continues, the force remains at a low level. Over a sequence of nonreinforced responses, there is a progressive elevation in the mean peak force. The standard deviations of the peak force distributions for each ordinal position (not shown) were correlated with the magnitude of the means.

The systematic changes in the peak force of response within the fixed-ratio cycle were consistently present over the 12 days of conditioning. They have also been observed for these and other subjects with sequences of six and 24 unreinforced responses. In general, the longer the unreinforced sequence, the

higher the level of force that was achieved. Conversely, longer reinforced sequences (with number of unreinforced responses held constant) tended to produce less extreme force elevation at the end of the cycle (see Fig. 1).

It is interesting to note that despite the frequent reinforcement of responses manifesting high peak forces (position I), a subsequent force decline persists. In the cycle positions following each reinforced response (II through 1) the force levels displayed are of the order of magnitude each animal produced during stable continuous reinforcement performance. The occurrence of reinforcement appears to set the occasion for an immediate return to this formerly reinforced level of responding. An earlier study (3) has shown that the Wistar rat's level of force emission can be brought under the control of an exteroceptive cue (presence or absence of light).

The control exerted by pellet delivery in the present study appears to be analogous to the cueing function served by the light. As was true of the light in the earlier study (3), pellet delivery during fixed-ratio reinforcement provides a stimulus condition following which a particular level of response force was formerly reinforced (during continuous reinforcement). It is therefore possible that the pellet delivery during fixed-ratio reinforcement provides a discriminative basis for the ob-

served force decline, effectively a return to the response force levels characteristic of continuous reinforcement performance.

The force elevation after nonreinforcement appears comparable to "an increase in the vigor of behavior immediately following non-reward," reported by Amsel (4) for the performance of rats in a double runway situation. Notterman and Block have shown that nonreinforced responses during discrimination training are characterized by higher forces than the reinforced responses (5). In the fixed-ratio schedule this increase in force provides a systematic correlate of cycle position. Ferster and Skinner (1) propose that the number of responses an organism has emitted during a fixed-ratio cycle comes to serve a discriminative function. Obviously, they are not referring to any "cognitive" operation of counting (6). It is possible that the "counting" under such circumstances reflects the animal's discrimination of its own level of force emission (7). Systematic variations in behavior such as have been demonstrated for force during fixed-ratio responding may well provide the basis for the cohesive nature of larger and more complicated behavioral units. Even in such an apparently homogeneous chain of responses as those which occur during this type of reinforcement, variations along a discriminable dimension (force) are found (8).

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8. This research was supported by the Office of Naval Research, under contract Nonr 1858 (19). The data are drawn from a portion of my Ph.D. dissertation (Columbia, 1961). The advice and encouragement of Drs. Joseph M. Notterman of Princeton University and Fred S. Keller, William J. McGill, and William N. Schoenfeld of Columbia University are gratefully acknowledged.

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