intermittent reinforcement which require a certain resistance to extinction to meet the reinforcement contingency have been reported for low levels of intermittency. Sidman and his co-workers (*3*) showed that cats could be trained on a fixed ratio of 8 (every eighth response was rewarded), but this degree of intermittency is small in comparison to the ratios of 100 or more that can be produced routinely with food reinforcement (Ferster and Skinner, *4*).

In view of the low resistance to extinction and the low levels of intermittency which have been reported when intracranial stimulation was used as a reward, it might appear that intracranial stimulation is a rather weak and ineffective reward. It is the purpose of this study to demonstrate that this is not necessarily the case.

Bipolar stainless steel electrodes, 0.25 mm in diameter, were implanted stereotaxically in 11 monkeys (*Macaca mulatta*). The Horsley-Clark coordinates (Labtronics instrument) were *A*-16, *L*-3, and $H + 3$, and were calculated to place the electrode tip in the vicinity of the medial forebrain bundle. Rectangular pulses, of 0.2 msec duration, 100 pulses per 0.5 second, were obtained from a Tektronix 162 waveform generator and 161 pulse generator which drove a cathode-follower output stage. The output from the cathode follower was fed to the electrodes through a General Radio isolation transformer. The current was monitored on a Tektronix 360 oscilloscope for each train of pulses, and the current usually employed was 3 ma.

After the operation, the monkeys were placed in a primate restraining chair and 2 days after the operation they were trained to press a telegraph key to obtain brain stimulation. After one session on a continuous reinforcement schedule (each lever press produced a brain stimulation), the animals were put onto a fixed ratio schedule, FR-5, which was gradually increased to FR-20. After at least a 1-hour session on FR-20, the current was turned off and the animal was allowed to work until the rate of lever pressing fell below 50 lever presses in a 15-minute period. In succeeding sessions intracranial stimulation reward was reinstated and the fixed ratio was increased to the point where postreinforcement pauses of several minutes were obtained.

Eight of the 11 monkeys learned to press the lever to obtain brain stimulation. All of these animals held a fixed ratio of 10, and seven of them held a ratio of 20. Four of the monkeys worked for brain stimulation on fixed ratios of 50 or greater. The highest ratio obtained was FR-150 in one monkey (Fig. 1*D*) and high rates of responding were maintained on this ratio for seven sessions which lasted a total of 20 hours. The effect on this monkey's behavior of increasing the ratio is illustrated in Fig. 1*A* to 1*D*.

In the extinction test, the four monkeys produced 342, 845, 4316, and 9129 responses before the extinction criterion was met. In subsequent experiments the highest ratios these animals would hold were FR-50, 100, 100, and 150 respectively. The extinction record for the FR-150 monkey is shown in Fig. 1*E*. The other four monkeys stopped responding after less than 100 lever presses when the current was turned off. None of these animals could be trained to hold a fixed ratio greater than 20. It appears that the length of the extinction record is indicative of the future fixed ratio performance of the animal for intracranial stimulation reward.

The results from half of the animals were consistent with previous reports in the literature in showing rather weak reinforcing properties of intracranial stimulation. However, the results from the other animals show that intracranial stimulation can maintain lever-pressing behavior on rather intermittent schedules of reinforcement and can generate a large number of extinction responses. These data are similar to those obtained by Ferster and Skinner (*4*) with food reinforcement. Thus, the data indicate that intracranial stimulation with a particular electrode placement can act as a strong and effective reinforcement.

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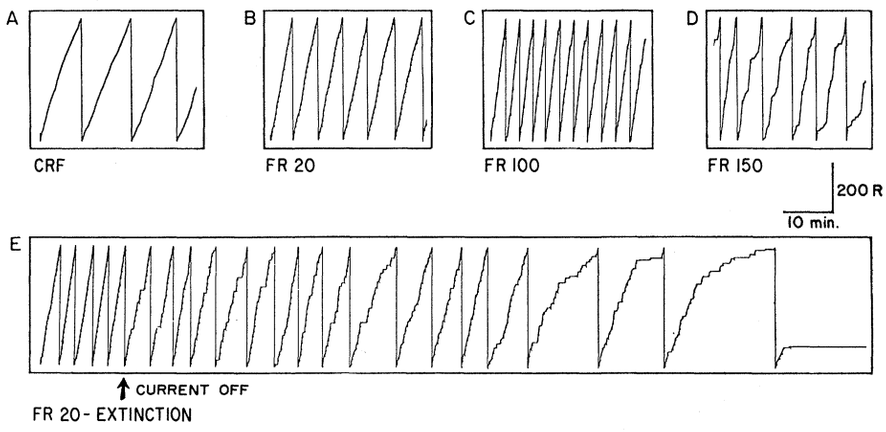

Fig. 1. Panels *A* through *D* show the effect of increasing the fixed ratio. The peak rate of 12,000 an hour was obtained on FR-100. At *D* with FR-150, a "strained ratio" was produced, characterized by long post-reinforcement pauses. Panel *E* illustrates the extinction record after a session on FR-20.

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18 November 1959

Heterogeneity of Human Ceruloplasmin

Abstract. Subfractionation of purified human ceruloplasmin, prepared from plasma of 9109 donors, has been carried out by chromatography on columns of diethylaminoethyl cellulose and hydroxylapatite. Electrophoretic analyses of these subfractions on starch gels, at *p*H 8.5 and 5.7, reveal the presence of at least four ceruloplasmins, two of which appear to differ in histidine content.

Hemoglobin (*1*), transferrin (*2*), haptoglobin (*3*), albumin (*4*), and isohemagglutinins of the proteins of human blood have each been shown to exist as two or more genetically determined molecular species. The electrophoretic analyses of Uriel (*5*) and the chromatographic separations of Broman and of Sankar (*6*) have indicated that human ceruloplasmin, the plasma copper protein, is also heterogeneous with respect to its molecular composition. By means of chromatography on calcium phosphate and diethylaminoethyl cellulose columns and by electrophoresis on starch gel we have been able to show that at least four different molecular species of ceruloplasmin are present in a purified preparation of this protein made from pooled plasma of 9109 donors (*7*).

The deep-blue preparation of ceruloplasmin used, CH-181 (*7*), contained 2.2 mg of protein, on the basis of nitrogen analysis, and 58.2 μg of copper per milliliter. To 3.0 ml of this ceruloplasmin solution was added 30.0 ml of a 0.05*M* phosphate buffer of *p*H 6.4.

All of the resulting solution was passed through a column of calcium phosphate (hydroxylapatite) (*8*). The ceruloplasmin was adsorbed and was visible as a blue band at the top of the column. After thorough washing with a 0.05*M* phosphate buffer of *p*H 6.4, elution with a 0.15*M* phosphate buffer of the same *p*H was carried out. The effluent, which was intensely blue, was termed fraction I. When measurements of the optical density of the effluent at 280 mμ indicated that no more protein was being eluted, a 0.4*M* phosphate buffer of *p*H 6.4 was run through the column. The effluent protein solution was intensely blue and was termed fraction II. After a second adsorption on, and elution from, calcium phosphate, each chromatographic fraction could be shown, by electrophoretic analysis of the type described below, to be substantially free of the other.

Fractions I and II, as solutions of the same optical density at 280 mμ, were compared with respect to copper content (*9*), blue color (*9*), oxidase activity toward paraphenylenediamine (*10, 11*) and exchangeability of protein-bound copper with ionic copper-64 (*11*). By these criteria the two fractions were indistinguishable, as is shown in Table 1.

We subfractionated fraction II, since we had found this made the electrophoretic distinction between the four types of ceruloplasmin clearer. Fraction II was diluted eightfold with water and adsorbed on a column of diethylaminoethyl cellulose (*12*) which had been previously equilibrated with a 0.05*M* phosphate buffer of *p*H 6.4. Elution with this same buffer, containing 0.15*M* NaCl, yielded a blue effluent, fraction II–1. When the effluent was free of ceruloplasmin, further elution with the same phosphate buffer, but now 0.30*M* in NaCl, resulted in a blue fraction II–2.

Vertical electrophoresis of fractions I, II–1, and II–2 was carried out, according to the method of Smithies (*13*), on a starch gel made with a borate buffer of 0.01 ionic strength, *p*H 8.5, and on a starch gel made with an acetate buffer of 0.01 ionic strength, *p*H 5.7. Fifty-microliter samples of each fraction were applied at the starting slits. A potential (of 170 volts for the borate gel, and 250 volts for the acetate gel) was applied for 19 hours at room temperature. On completion of the run, each gel was sliced into two layers, and strips of heavy filter paper, saturated with a solution of 0.5 percent paraphenylenediamine dihydrochloride in an acetate buffer of ionic strength 1.0, *p*H 5.1, were placed on the cut surfaces for 1 minute. The gels were placed in a closed, moist chamber and allowed to stand for 1 to 3 hours at room temperature. The appearance of dark purple bands indicated the sites to which the ceruloplasmins had migrated.

Table 1. Comparative chemistry of solutions of chromatographically separated fractions I and II of ceruloplasmin CH–181.

Relative protein concentration ($O.D._{280}^{1\,cm}$)	Copper content (*9*) (μg/ml)	Blue color (*9*) ($\Delta O.D._{610}^{1\,cm}$)	Enzymatic activity (*10,11*) ($O.D._{530}^{1\,cm}$/min)*	Cu^{64} exchange (*11*)
		Fraction I		
0.468	0.83	0.0196	0.0258	Yes
		Fraction II		
0.465	0.85	0.0198	0.0258	Yes

* Based on oxidative darkening of paraphenylenediamine, which is catalyzed by ceruloplasmin.

Figure 1 shows that according to the electrophoretic analyses carried out at *p*H 8.5, fractions I and II–1 each consist of one component. Electrophoresis of fraction II–2 at *p*H 8.5, however, revealed three ceruloplasmin bands with the ceruloplasmin of intermediate mobility constituting the greater part of the oxidase activity of this fraction. That these five bands represent four different ceruloplasmins is seen when the results of the experiments run at *p*H 5.7 are also considered. Thus, first, fraction I and fraction II–1, which could not be distinguished at *p*H 8.5, have grossly different mobilities at *p*H 5.7. Second, the component of fraction II–2 which, according to its mobility at *p*H 8.5, could be either fraction I or fraction II–1 is seen, by its behavior at *p*H 5.7, to be fraction II–1 which had not been completely removed from fraction II–2. The four ceruloplasmins are, therefore, fraction I, fraction II–1, and the two slowest components of fraction II–2. To simplify the nomenclature, we propose to call the ceruloplasmin of fraction I, which preliminary observations have shown to be the largest single component of almost all individual sera, C; that of fraction II–1, D_0; and the two slowest components of fraction II–2, D_1 and D_2, in order of decreasing mobility at *p*H 8.5. These terms are closely, although not exactly, analogous to those chosen by Smithies (*2*) for the human transferrins.

Similar electrophoretic analyses were performed on ceruloplasmin solutions prepared from ten individual sera only by means of the mild treatment involved in chromatography on diethylaminoethyl cellulose (*12*). The results, which also indicated that the different

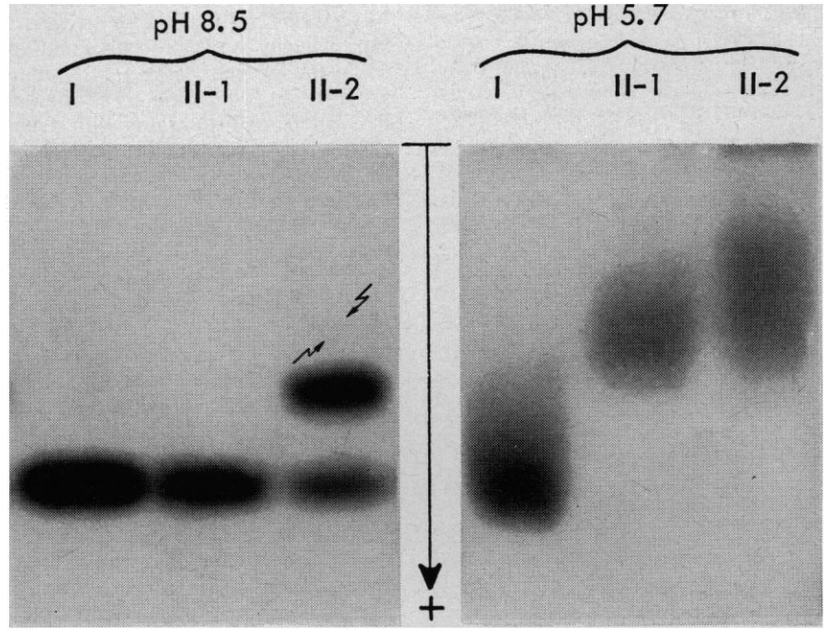

Fig. 1. Electrophoresis at *p*H 8.5 and 5.7, on starch gels, of ceruloplasmin fractions I, II–1, and II–2. The starting slits are located at the level of the horizontal line at the top of the vertical arrow, which points in the direction of migration, toward the anode. The position of the faint third band of fraction II–2, at *p*H 8.5, is indicated by the jagged arrows. At *p*H 5.7 there is considerable trailing of the ceruloplasmins. The greater fuzziness of the bands at this *p*H may, however, also be due in part to further unresolved heterogeneity, particularly in fraction II–2.

mobilities discussed above are not artifacts of the fractionating procedures (*7*) used to prepare ceruloplasmin CH-181, showed that eight of these individual preparations contained at least three ceruloplasmins and two of them contained at least two ceruloplasmins. In view of these experiments on individual sera, it seems probable that the differences in electrophoretic mobility that we have demonstrated in the four ceruloplasmins derived from pooled plasma are due to differences in the content of charged amino acid residues in the native ceruloplasmins. Such a correlation between mobility and amino acid composition has been found for several of the human hemoglobins (*1*). Indeed, our results suggest that ceruloplasmin D_0 possesses a greater number of histidine residues than ceruloplasmin C, since (i) both ceruloplasmins have virtually the same mobility at *p*H 8.5 but ceruloplasmin D_0 is distinctly slower than ceruloplasmin C at *p*H 5.7; (ii) both ceruloplasmins are anionic at each of these *p*H values (*9*); and (iii) histidine is the only charged amino acid residue titrated appreciably between *p*H 8.5 and 5.7 (*14*). On the basis of what is known of hemoglobin, it appears that such differences in structure in ceruloplasmin are likely to be genetic in origin (*15*).

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Isolation of a Bacteriophage Specific for the F+ and Hfr Mating Types of Escherichia coli K-12

Abstract. Seven phage stocks have been prepared from a sample of sewerage. The stocks grow only on the F+ and Hfr mating types of *Escherichia coli* strain K-12. This specificity is not shared by any previously characterized phage which was tested. The specificity of one of the isolates, f1, has been well correlated with the salmonella cross-streak test described by Zinder.

The bacteria *Escherichia coli* have been shown to consist of three mating types: F−, F+, and Hfr (*1, 2*). F− strains serve as recipients for genetic material donated by F+ or Hfr strains during conjugation. F+ and Hfr strains differ in two respects: First, under proper selective conditions Hfr strains display a higher frequency of recombination with F− strains than do F+ strains. Second, F+ strains possess a highly infective F agent capable of converting F− strains to the F+ mating type. This agent is not detectable in Hfr strains. Thus, matings of Hfr and F− strains result in F− progeny (or Hfr progeny under certain conditions), while the progeny of F+, F− matings are F+. It is possible to eliminate the F agent from F+ strains with chemical agents, thus converting these bacteria to the F− mating type (*3*).

There are also differences in the physiology of F+ (or Hfr) and F− bacterial cells. F+ cells show a greater tendency to autoagglutinate, agglutinate at a higher *p*H, and are more easily stained with acid dyes than the corresponding F− cells (*4*). Furthermore, Zinder has described a "staining" interaction between *E. coli* and *Salmonella typhimurium* and between *E. coli* and *E. coli* which distinguishes F− from F+ or Hfr bacterial mating types (*5*).

In view of the above observations, it seemed reasonable to search for a bacteriophage capable of distinguishing F− from F+ and Hfr mating types. This report describes the isolation and testing of such a phage.

The following mutant strains of *E. coli* K-12 were used [prepared as described by Lederberg (*1*)]: 58-161 (F+), Hfr (Cavalli), and Y-10 (F−). The pertinent characteristics of these strains are as follows: 58-161 and Hfr (Cavalli) are capable of lactose fermentation (lac+) but are unable to grow in the absence of methionine (meth−); Y-10 is incapable of lactose fermentation (lac−) and requires threonine, leucine, and thiamine for growth (thr−, leu−, thi−). Two strains of salmonella were used: *S. typhimurium* LT 2 and LT 2 F+ (LT 2 infected with the F agent of *E. coli* K-12 (*5*). Other bacterial strains which were used are described in the text where appropriate. Various bacteriophages were used and are described in the text where appropriate.

The media used have been described by Lederberg (*1*). In particular broth agar, minimal agar, and eosin methylene blue agar with lactose (EMB lac) and without sugar (EMB 0) were employed.

Phage plating was done by the method of Adams (*6*). Also cross-streaking of bacteria with phage on EMB 0 provided a sensitive test for bacterial susceptibility.

The mating type of a bacterial strain was determined by mixing the strain with F− bacteria auxotrophic for nutrients complementary to the deficiencies of the strain being tested. The production of colonies by this mixture when spread on minimal agar indicated the mating type to be either F+ or Hfr (*1*).

Mating types were also determined by cross-streaking against *S. typhimurium* LT 2 on EMB 0 following the method of Zinder (*5*).

In order to isolate a phage with the required characteristics it was necessary to obtain two strains of *E. coli* K-12 identical in all respects but mating type. To accomplish this it was decided to "cure" an F+ strain by the method of Hirota (*3*). Acriflavine was added to a saturated broth culture of 58-161 (F+) to a final concentration of 1 mg/ml. After 2 hours at 37°C, the culture was washed and streaked on broth agar. A clone (labeled 58-161 F−) was isolated which was incapable of colony production when mixed with Y-10 (F−) on minimal agar (*1*). Clones 58-161 and 58-161 F− were found to be identical in all respects other than mating type.

To test the possibility that some previously isolated phage would distinguish mating types, the following phages were spotted on plates seeded with 58-161 or 58-161 F−: T1, T2, T3, T4, T5, T6, T7, C16, lambdal, P22, P22V, phi, P2, and megatherium T. An identical pattern of susceptibility and resistance was displayed by the two strains.

To isolate and characterize a phage that grows only on F+ and Hfr strains of *E. coli* K-12, sewer water was centrifuged and sterilized with chloroform; nutrient broth was added to preserve phage. Forty-eight plaques were isolated from a plating of this solution on 58-161 F− and 90 plaques from plates seeded with Y-10 (F−). The spotting of these