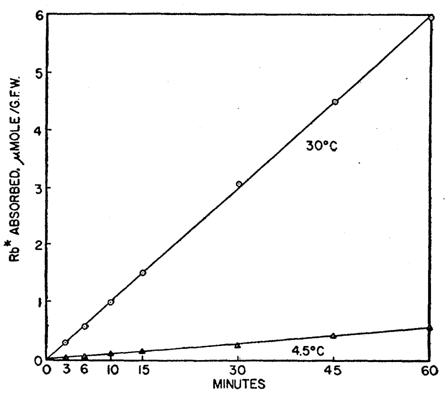

Fig. 1. Absorption of rubidium by barley roots as a function of time and temperature. Roots, 1.00 g solution: 0.10 mmole/lit. of $Rb^{86}Cl$, 0.50 mmole/lit. of $CaCl_2$, *p*H 5.6, aerated.

ion exchange, and their initial penetration into the tissue is by diffusion into the "outer" (*3*) or "water free" (*4*) space. On the basis of such findings, a generalized diagram has been presented by Briggs, Hope, and Robertson (*5*) according to which the initial uptake of the cation of a salt exceeds that of the anion and its initial equilibration is much slower than that of the anion. After this initial period, both the cation and the anion are absorbed by the active transport mechanisms, at steady rates.

Figure 1 shows that when calcium is present—the physiological condition (*1*)—there is no more evidence for an appreciable period of equilibration before the onset of steady-state, metabolically mediated absorption of rubidium than there is with anions. The first measurement was made 3 minutes after the start of the absorption period. At 30°C the rate of absorption for the first 3 minutes was 0.10 μmole per gram per minute—the same rate that was maintained for the full 60-minute absorption period. Absorption even during the first few minutes is fully temperature-sensitive; that is, it is metabolically mediated and does not represent "outer" space equilibration, which evidently occurs extremely rapidly (see *6*). It is concluded that when calcium at physiological concentrations is present, the general, nonselective cation-exchange capacity of the tissue is largely satisfied by calcium ions and the time course of the absorption of monovalent cations under these physiological conditions is precisely like that of anions. Overt, nonselective cation exchange plays no role, and the diagram given by Briggs *et al.* (*5*) is not a valid general representation of the time course of the absorption of monovalent cations.

We want to point out parenthetically that there is no evidence, under these physiological conditions (which include the presence of calcium in the solutions), of measurable instantaneous adsorption (zero-time intercepts) ostensibly reflecting the concentrations of the specific ion-carrying sites (*7*). In our experiments zero-time intercepts of the order specified would be readily apparent.

Kinetic studies (*8*) have resulted in the following model of ion transport across cellular membranes. The ion combines with a carrier which resides in and is part of a membrane not permeable with respect to free ions (ions not combined with a carrier):

$$R + M_{\text{outside}} \underset{k_2}{\overset{k_1}{\rightleftarrows}} RM$$

where *R* is the carrier and *M* is the ion. Once the carrier-ion complex *RM* has reached the far side of the membrane, the ion dissociates from the carrier into the "inner" (transmembrane) compartment or space, as a result of a chemical change in the carrier:

$$RM \underset{k_4}{\overset{k_3}{\rightleftarrows}} R' + M_{\text{inside}}$$

The model is analogous to the mechanism of enzyme action:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES; \; ES \underset{k_4}{\overset{k_3}{\rightleftarrows}} E + P$$

Michaelis-Menten enzyme kinetics are based on the postulate that the rate of the reaction is proportional to the concentration of enzyme-substrate complex *ES*. When both k_2 and k_4 are appreciable, the net rate of the reaction depends not only on the concentration of the enzyme-substrate complex but on the concentration of product *P* as well, and it decreases as the concentration of product rises. In work with reversible enzyme reactions this effect is avoided by measuring the "initial rate" of the enzyme reaction, before the concentration of product reaches an appreciable value.

In carrier kinetics, the rate of transport is considered proportional to the concentration of carrier-ion complex *RM*, in analogy with enzyme-mediated catalysis, and "M_{inside}"—that is, absorbed ions—corresponds to "product" (*8*). However, in our experiment, the concentration of "product"—that is, ions absorbed—does not remain negligible for any length of time (Fig. 1). Within less than 1 minute the internal concentration of Rb rises to a value well above the concentration in the external solution. Despite the subsequent buildup of internal concentrations far in excess of the external concentration, the rate of absorption does not slacken; that is, the rate is independent of the rising internal concentrations of Rb ("product"). This means, in terms of the foregoing hypothesis, that the overall process is to a marked degree irreversible. It is recognized that the internal concentration will eventually reach a level at which the rate of absorption will slow down. The experiment shows, however, that the equilibrium is far to the right—that is, either k_1 *or* k_3 (or both) is greatly in excess of the corresponding reverse rate. Other, independent evidence (*9*) bears out this conclusion concerning the high degree of irreversibility of the absorption process (*10*).

EMANUEL EPSTEIN
D. W. RAINS
WALTER E. SCHMID

Department of Soils
and Plant Nutrition,
University of California, Davis

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Formation of Double Hydroxides and the Titration of Clays

When increasing amounts of MgO are added to a known quantity of Al^{3+} saturated Wyoming bentonite in a dilute salt solution, the resulting titration curve shows two buffering regions, one below and the other above *p*H 7.0. The latter occurs at a *p*H considerably below that at which the precipitation of $Mg(OH)_2$ would be expected, and our report deals exclusively with it. Similar buffering effects, not confined to MgO, have been referred to as an abnormal uptake of the cations of the base by the clay at *p*H's just below those of the saturated solutions of the hydroxide (*1*). It is our purpose to suggest, with supporting experimental evidence, another possible explanation for this phenomenon when MgO is the base used.

Table 1. Solid phases present during titration of a number of salts with MgO, as shown by x-ray diffraction of oriented samples.

Salt	Steep part of titration curve	End of titration
$Mg(NO_3)_2$	No precipitate.	$Mg(OH)_2$
$La(NO_3)_3$	$La(OH)_3$	$La(OH)_3$ and $Mg(OH)_2$
$Al(NO_3)_3$	X-ray amorphous	Double hydroxide
$Fe(NO_3)_3$	X-ray amorphous	Double hydroxide

It is known that Mg^{2+} forms relatively insoluble double hydroxides with either Al^{3+} or Fe^{3+} (*2*) but not with La^{3+} (*3*). It seemed possible therefore that the buffering above *p*H 7.0, described in the previous paragraph, could be caused by the precipitation of the double hydroxide of Al^{3+} and Mg^{2+}. If this were so, the corresponding titration curve for a clay saturated with Fe^{3+} might also show a buffering zone below the *p*H at which Mg $(OH)_2$ is precipitated whereas the curves for clays saturated with La^{3+} and Mg^{2+} should not. With this in mind we made the following experiments.

Four 4-gram samples of Wyoming bentonite were saturated with Al^{3+}, Fe^{3+}, La^{3+}, and Mg^{2+}, respectively. Each was suspended in 150 ml of $10^{-3}M$ $MgCl_2$ in Lucite reaction vessels and titrated with MgO at 25°C. After each increment of MgO was added the suspensions were stirred for 24 hours if below, and 48 hours if above *p*H 7.0, before the final *p*H measurement was made. The stirring was accomplished by bubbling CO_2-free air through the suspensions, which also ensured CO_2-free systems. Figure 1 shows that buffering occurred at about *p*H 8.7 and 9.2 respectively for the clays saturated with Al^{3+} and Fe^{3+} and at *p*H 10.0 for those saturated with La^{3+} and Mg^{2+}. Since the solubility product for precipitated Mg $(OH)_2$ is about 10^{-11} (*4*), its formation should have occurred at about *p*H 10 in these systems. Thus it appears that the buffering with clays saturated with La^{3+} and Mg^{2+} was caused by the precipitation of $Mg(OH)_2$, but that this reaction does not account for the behavior of the clays saturated with Fe^{3+} and Al^{3+}.

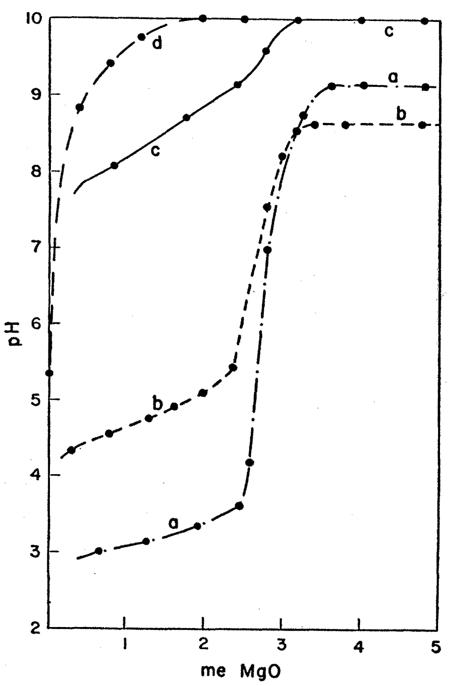

Fig. 1. Titration of Wyoming bentonite with MgO. *a*, Fe^{3+} saturated; *b*, Al^{3+} saturated; *c*, La^{3+} saturated; *d*, Mg^{2+} saturated.

In order to determine the characteristics of the titration curves of solutions of the four cations, titrations were made with MgO as described above but in the absence of clays. Each solution contained 2.4 meq of the appropriate cation as the nitrate salt in a 150-ml volume. The resulting curves (Fig. 2) were strikingly similar to the corresponding clay titration curves in Fig. 1. Since $Mg(OH)_2$ should have precipitated at about *p*H 9.7 in these solutions, it apparently caused the buffering in the Mg^{2+} and La^{3+} solutions. With the Al^{3+} and Fe^{3+} solutions, as with the clay systems, however, the buffering occurred at lower *p*H's, and lower with Al^{3+} than with Fe^{3+}.

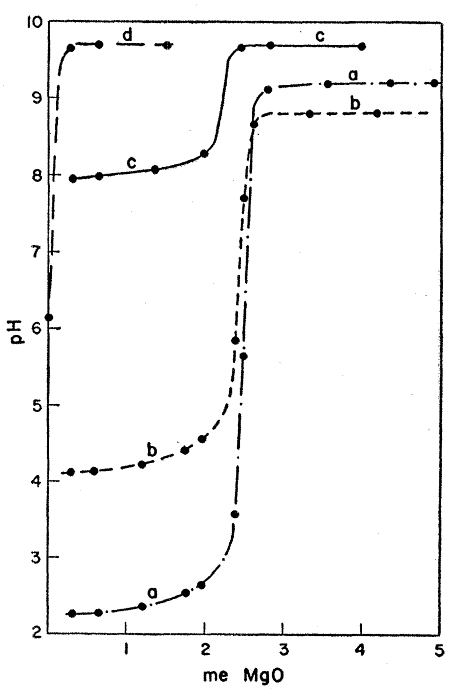

Fig. 2. Titration of nitrate salts with MgO. *a*, $Fe(NO_3)_3$; *b*, $Al(NO_3)_3$; *c*, $La(NO_3)_3$; and *d*, $Mg(NO_3)_2$.

With the clay systems, x-ray diffraction could not be used to identify the precipitates formed because of the clay present. It was possible to do this, however, when the nitrate solutions were titrated. Samples were taken when the steep part of the curves had been reached and again at the end of the titrations, and specimens were made by drying portions on glass slides. Table 1 shows the results obtained. With the $Mg(NO_3)_2$ solution there was, of course, no precipitation before the flat part of the curve at *p*H 9.7 was reached, but $Mg(OH)_2$ (brucite) was present at the end of the titrations. $La(OH)_3$ was the only precipitate formed from $La(NO_3)_3$ when the steep part of the curve was reached, and at the end of the titration both $La(OH)_3$ and $Mg(OH)_2$ were present. With $Al(NO_3)_3$ and $Fe(NO_3)_3$ the first samples were amorphous to x-rays while the second were similar to those described (*2*) for the double hydroxides of Mg^{2+} and Al^{3+} or Fe^{3+}—that is, they showed an integral series of basal spacings near 9.7 A. There was no evidence of brucite in these latter samples. Therefore it was concluded that the formation of double hydroxides was responsible for the buffering zone at a *p*H below that expected for the precipitation of $Mg(OH)_2$ in the nitrate solutions.

Even though double hydroxides were not identified in the clay suspensions, the similarity of the titration curves in Figs. 1 and 2 is rather convincing evidence that their formation is involved in the titration with MgO of clays saturated with Al^{3+} and Fe^{3+} (*5*).

R. C. TURNER
J. E. BRYDON

Soil Research Institute,
Canada Department of Agriculture,
Ottawa, Ontario

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4 December 1961

Method for Studying Exploratory Behavior in Infants

Abstract. An experimental crib has been designed for use in studying the effect of visual feedback upon the exploratory behavior of the human infant. It can be easily adapted to older children, to some animals, and to problems other than the development of exploratory behavior.

Exploratory behavior is clearly apparent in human infants by the time they are 3 months old (*1*). An apparatus designed for use in studying the beginnings of this behavior, especially in relation to feedback from the external environment, is also adaptable to the study of other kinds of early behavior, both human and animal. The apparatus holds the infant in a suitable position, permits measurement of certain behavior, and provides for sensory feedback from that behavior.

A specially designed crib is approximately 5 feet long, 3 feet wide, and 4 feet high. It stands on legs 2 feet 5 inches long. Interior surfaces are made of Acoustitex nonperforated sound-insulation boards, painted ivory. Six 6-watt bulbs are mounted at the top of the wall behind the infant. The sides of the crib are double sliding panels. Behind the infant is a double observation window, 28 inches long by 18 inches wide, glass on the outside and plastic on the inside. At the other end is a double glass window, 16 by 17 inches, set at an angle of 60°, through which images can be projected on a screen. The screen is frosted Plexiglas, 34 by 31 inches, also set at an angle of 60°. The crib is ventilated by a blower of capacity 60 ft^3/min (Fig. 1).

The infant faces the screen at a distance of 30 inches, supported in a commercially available canvas seat (*2*), on a modified frame adjustable at angles between 45° and 90°. A cloth strap holds the infant across the chest and abdomen, leaving its hands and feet

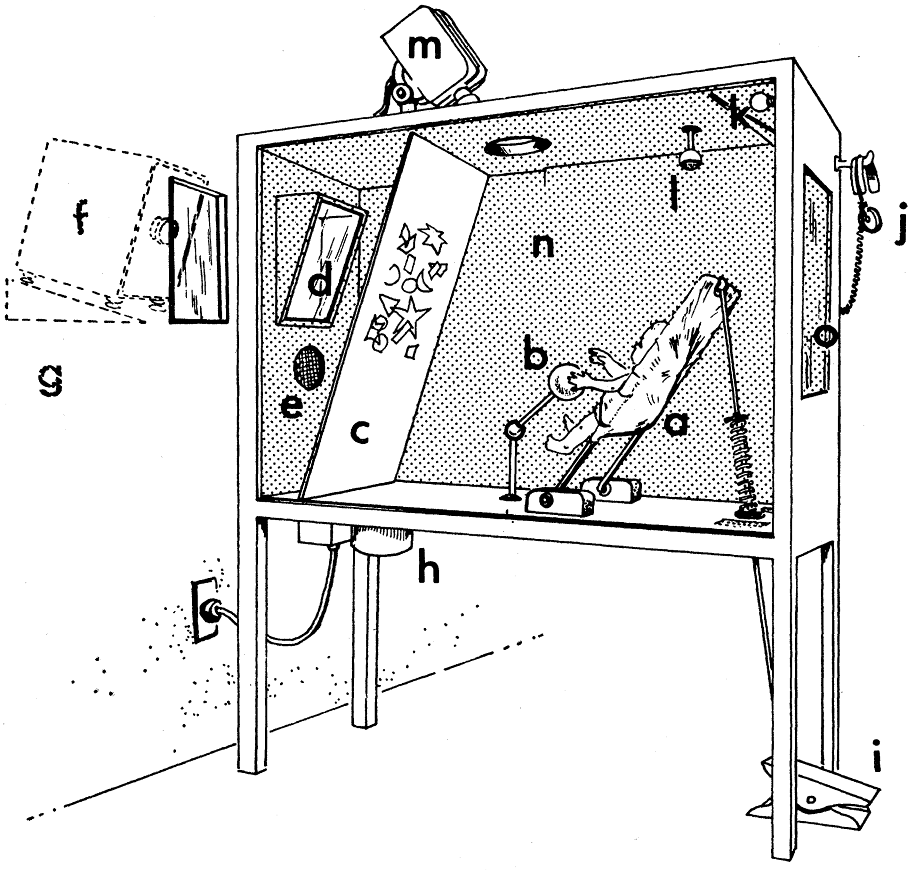

Fig. 1. Experimental crib: *a*, seat; *b*, manipulandum; *c*, screen; *d*, projection opening; *e*, sound source; *f*, projector; *g*, control room; *h*, ventilator; *i*, rocker; *j*, intercom; *k*, crib lights; *l*, microphone; *m*, television camera; *n*, doors of crib; *o*, window.

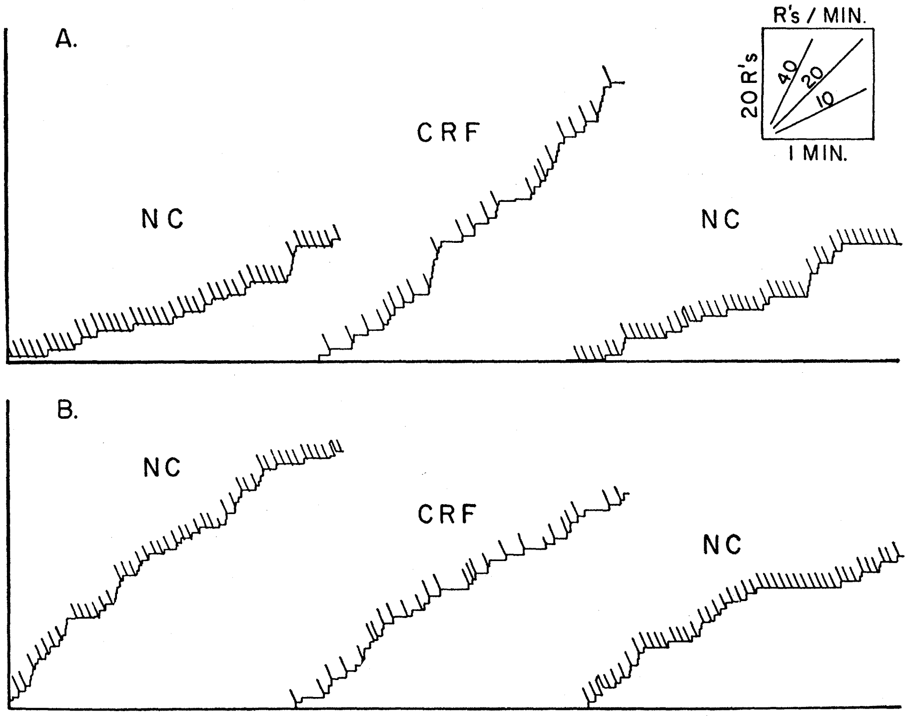

Fig. 2. Cumulative response curves for two infants, showing (*A*) performance sensitive to changes in experimental conditions and (*B*) performance not sensitive. Under the noncontingent (*NC*) condition the movie appeared for alternate 1½-second periods regardless of the infant's behavior, under the continuous reinforcement (*CFR*) condition the movie followed each of the infant's responses.