CURRENT PROBLEMS IN RESEARCH

Affectional Responses in the Infant Monkey

Orphaned baby monkeys develop a strong and persistent attachment to inanimate surrogate mothers.

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Investigators from diverse behavioral fields have long recognized the strong attachment of the neonatal and infantile animal to its mother. Although this affectional behavior has been commonly observed, there is, outside the field of ethology, scant experimental evidence permitting identification of the factors critical to the formation of this bond. Lorenz (1) and others have stressed the importance of innate visual and auditory mechanisms which, through the process of imprinting, give rise to persisting following responses in the infant bird and fish. Imprinting behavior has been demonstrated successfully in a variety of avian species under controlled laboratory conditions, and this phenomenon has been investigated systematically in order to identify those variables which contribute to its development and maintenance [see, for example, Hinde, Thorpe, and Vince (2), Fabricius (3), Hess (4), Jaynes (5), and Moltz and Rosenblum (6)]. These studies represent the largest body of existent experimental evidence measuring the tie between infant and mother. At the mammalian level there is little or no systematic experimental evidence of this nature.

Observations on monkeys by Carpenter (7), Nolte (8), and Zuckermann (9) and on chimpanzees by Kohler (10) and by Yerkes and Tomlin (11) show that monkey and chimpanzee infants develop strong ties to their mothers and that these affectional attachments may persist for years. It is, of course, common knowledge that human infants form strong and persistent ties to their mothers.

Although students from diverse scientific fields recognize this abiding attachment, there is considerable disagreement about the nature of its development and its fundamental underlying mechanisms. A common theory among psychologists, sociologists, and anthropologists is that of learning based on drive reduction. This theory proposes that the infant's attachment to the mother results from the association of the mother's face and form with the alleviation of certain primary drive states, particularly hunger and thirst. Thus, through learning, affection becomes a self-supporting, derived drive (12). Psychoanalysts, on the other hand, have stressed the importance of various innate needs, such as a need to suck and orally possess the breast (2), or needs relating to contact, movement, temperature (13), and clinging to the mother (14).

The paucity of experimental evidence concerning the development of affectional responses has led these theorists to derive their basic hypotheses from deductions and intuitions based on observation and analysis of adult verbal reports. As a result, the available observational evidence is often forced into a preconceived theoretical framework. An exception to the above generalization is seen in the recent attempt by Bowlby (14) to analyze and integrate the available observational and experimental evidence derived from both human and subhuman infants. Bowlby has concluded that a theory of component instinctual responses, species specific, can best account for the infant's tie to the mother. He suggests that the species-specific responses for human beings (some of these responses are not strictly limited to human beings) include contact, clinging, sucking, crying, smiling, and following. He further emphasizes that these responses are manifested independently of primary drive reduction in human and subhuman infants.

The absence of experimental data which would allow a critical evaluation of any theory of affectional development can be attributed to several causes. The use of human infants as subjects has serious limitations, since it is not feasible to employ all the experimental controls which would permit a completely adequate analysis of the proposed variables. In addition, the limited response repertoire of the human neonate severely restricts the number of discrete or precise response categories that can be measured until a considerable age has been attained. Thus, critical variables go unmeasured and become lost or confounded among the complex physiological, psychological, and cultural factors which influence the developing human infant.

Moreover, the use of common laboratory animals also has serious limitations, for most of these animals have behavioral repertoires very different from those of the human being, and in many species these systems mature so rapidly that it is difficult to measure and assess their orderly development. On the other hand, subhuman primates, including the macaque monkey, are born at a state of maturity which makes it possible to begin precise measurements within the first few days of life. Furthermore, their postnatal maturational rate is slow enough to permit precise assessment of affectional variables and development.

Over a 3-year period prior to the beginning of the research program reported...
here (13), some 60 infant macaque monkeys were separated from their mothers 6 to 12 hours after birth and raised at the primate laboratory of the University of Wisconsin. The success of the procedures developed to care for these neonates was demonstrated by the low mortality and by a gain in weight which was approximately 25 percent greater than that of infants raised by their own mothers. All credit for the success of this program belongs to van Wagenen (16), who had described the essential procedures in detail.

These first 3 years were spent in devising measures to assess the multiple capabilities of the neonatal and infantile monkey. The studies which resulted have revealed that the development of perception, learning, manipulation, exploration, frustration, and timidity in the macaque monkey follows a course and sequence which is very similar to that in the human infant. The basic differences between the two species appear to be the advanced postnatal maturational status and the subsequent more rapid growth of the infant macaque. Probably the most important similarities between the two, in relation to the problem of affectional development, are characteristic responses that have been associated with, and are considered basic to, affection; these include nursing, clinging, and visual and auditory exploration.

In the course of raising these infants we observed that they all showed a strong attachment to the cheesecloth blankets which were used to cover the wire floors of their cages. Removal of these cloth blankets resulted in violent emotional behavior. These responses were not short-lived; indeed, the emotional disturbance lasted several days, as was indicated by the infant's refusal to work on the standard learning tests that were being conducted at the time. Similar observations had already been made by Foley (17) and by van Wagenen (16), who stressed the importance of adequate contact responses to the very survival of the neonatal macaque. Such observations suggested to us that contact was a true affectional variable and that it should be possible to trace and measure the development and importance of these responses. Indeed there seemed to be every reason to believe that one could manipulate all variables which have been considered critical to the development of the infant's attachment to a mother, or mother surrogate.

To attain control over maternal variables, we took the calculated risk of constructing and using inanimate mother surrogates rather than real mothers. The cloth mother that we used was a cylinder of wood covered with a sheet of terry cloth (18), and the wire mother was a hardware-cloth cylinder. Initially, sponge rubber was placed underneath the terry cloth sheath of the cloth mother surrogate, and a light bulb behind each mother surrogate provided radiant heat. For reasons of sanitation

**Fig. 1.** Wire and cloth mother surrogates.

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![Graph](image-url)

**Time spent on cloth and wire mother surrogates.** Fig. 2 (left). Short term. Fig. 3 (right). Long term.

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and safety these two factors were eliminated in construction of the standard mothers, with no observable effect on the behavior of the infants. The two mothers were attached at a 45-degree angle to aluminum bases and were given different faces to assure uniqueness in the various test situations (Fig. 1). Bottle holders were installed in the upper middle part of the bodies to permit nursing. The mother was designed on the basis of previous experience with infant monkeys, which suggested that nursing in an upright or inclined position with something for the infant to clasp facilitated successful nursing and resulted in healthier infants (see 16). Thus, both mothers provided the basic known requirements for adequate nursing, but the cloth mother provided an additional variable of contact comfort. That both of these surrogate mothers provided adequate nursing support is shown by the fact that the total ingestion of formula and the weight gain was normal for all infants fed on the surrogate mothers. The only consistent difference between the groups lay in the softer stools of the infants fed on the wire mother.

Development of Affectional Responses

The initial experiments on the development of affectional responses have already been reported (19) but will be briefly reviewed here, since subsequent experiments were derived from them. In the initial experiments, designed to evaluate the role of nursing on the development of affection, a cloth mother and a wire mother were placed in different cubicles attached to the infant's living cage. Eight newborn monkeys were placed in individual cages with the surrogates; for four infant monkeys the cloth mother lactated and the wire mother did not, and for the other four this condition was reversed.

The infants lived with their mother surrogates for a minimum of 165 days, and during this time they were tested in a variety of situations designed to measure the development of affectional responsiveness. Differential affectional responsiveness was initially measured in terms of mean hours per day spent on the cloth and on the wire mothers under two conditions of feeding, as shown in Fig. 2. Infants fed on the cloth mother and on the wire mother have highly similar scores after a short adaptation period (Fig. 3), and over a 165-day period both groups show a distinct preference for the cloth mother. The persistence of the differential responsiveness to the mothers for both groups of infants is evident, and the over-all differences between the groups fall short of statistical significance.

These data make it obvious that contact comfort is a variable of critical importance in the development of affectional responsiveness to the surrogate mother, and that nursing appears to play a negligible role. With increasing age and opportunity to learn, an infant fed from a lactating wire mother 'does not become more responsive to her, as would be predicted from a derived-drive theory, but instead becomes increasingly more responsive to its nonlactating cloth mother. These findings are at complete variance with a drive-reduction theory of affectional development.

The amount of time spent on the mother does not necessarily indicate an affectional attachment. It could merely reflect the fact that the cloth mother is a more comfortable sleeping platform or a more adequate source of warmth for the infant. However, three of the four infants nursed by the cloth mother and one of the four nursed by the wire mother left a gauze-covered heating pad that was on the floor of their cages during the first 14 days of life to spend up to 18 hours a day on the cloth mother. This suggests that differential heating or warmth is not a critical variable within the controlled temperature range of the laboratory.

Other tests demonstrate that the cloth mother is more than a convenient nest; indeed, they show that a bond develops between infant and cloth-mother surrogate that is almost unbelievably similar to the bond established between human mother and child. One highly definitive test measured the selective maternal responsiveness of the monkey infants under conditions of distress or fear.

Various fear-producing stimuli, such as the moving toy bear illustrated in Fig. 4, were presented to the infants in their home cages. The data on differential responses under both feeding conditions are given in Fig. 5. It is apparent that the cloth mother was highly preferred to the wire mother, and it is a fact that these differences were unrelated to feeding conditions—that is, nursing on the cloth or on the wire mother. Above and beyond these objective data are observations on the form of the infants' responses in this situation. In spite of their abject terror, the infant monkeys, after reaching the cloth mother and rubbing their bodies about hers, rapidly come to lose their fear of the frightening stimuli. Indeed, within a minute or two most of the babies were visually exploring the very thing which so shortly before had seemed an object of evil. The bravest of the babies would actually leave the mother and approach the fearful monsters, under, of course, the protective gaze of their mothers.

These data are highly similar, in terms of differential responsiveness, to the time scores previously mentioned and indicate the overwhelming importance of contact comfort. The results are so striking as to suggest that the primary function of nursing may be that of insuring frequent and intimate contact between mother and infant, thus facilitating the localization of the source of contact comfort. This interpretation finds some support in the test discussed above. In both situations the infants...
nursed by the cloth mother developed consistent responsiveness to the soft mother earlier in testing than did the infants nursed by the wire mother, and during this transient period the latter group was slightly more responsive to the wire mother than the former group. However, these early differences shortly disappeared.

Additional data have been obtained from two groups of four monkeys each which were raised with a single mother placed in a cubicule attached to the living-cage. Four of the infants were presented with a lactating wire mother and the other four were presented with a nonlactating cloth mother. The latter group was hand-fed from small nursing bottles for the first 30 days of life and then weaned to a cup. The development of responsiveness to the mothers was studied for 165 days; after this the individual mothers were removed from the cages and testing was continued to determine the strength and persistence of the affectional responses.

Figure 6 presents the mean time per day spent on the respective mothers over the 165-day test period, and Fig. 7 shows the percentage of responses to the mothers when a fear-producing stimulus was introduced into the home cage. These tests indicate that both groups of infants developed responsiveness to their mother surrogates. However, these measures did not reveal the differences in behavior that were displayed in the reactions to the mothers when the fear stimuli were presented. The infants raised on the cloth mother would rush to the mother and cling tightly to her. Following this initial response these infants would relax and either begin to manipulate the mother or turn to gaze at the feared object without the slightest sign of apprehension. The infants raised on the wire mother, on the other hand, rushed away from the feared object toward their mother but did not cling to or embrace her. Instead, they would either clutch themselves and rock and vocalize for the remainder of the test or rub against the side of the cubicule. Contact with the cubicule or the mother did not reduce the emotionality produced by the introduction of the fear stimulus. These differences are revealed in emotionality scores, for behavior such as vocalization, crouching, rocking, and sucking, recorded during the test. Figure 8 shows the mean emotionality index for test sessions for the two experimental groups, the dual-mother groups, and a comparable control group raised under standard laboratory conditions. As can be seen, the infants raised with the single wire mother have the highest emotionality scores of all the groups, and the infants raised with the single cloth mother or with a cloth and wire mother have the lowest scores. It appears that the responses made by infants raised only with a wire mother were more in the nature of simple flight responses to the fear stimulus and that the presence of the mother surrogate had little effect in alleviating the fear.

During our initial experiments with the dual-mother conditions, responsiveness to the lactating wire mother in the fear tests decreased with age and opportunity to learn, while responsiveness to the nonlactating cloth mother increased. However, there was some indication of a slight increase in frequency of response to the wire mother for the first 30 to 60 days (see Fig. 5). These data suggest the possible hypothesis that nursing facilitated the contact of infant and mother during the early developmental periods.

The interpretation of all fear testing is complicated by the fact that all or most "fear" stimuli evoke many positive exploratory responses early in life and do not consistently evoke flight responses until the monkey is 40 to 50 days of age. Similar delayed maturation of visually induced fear responses has been reported for birds (3), chimpanzees (20), and human infants (21).

Because of apparent interactions between fearful and affectional developmental variables, a test was designed to trace the development of approach and avoidance responses in these infants. This test, described as the straight-alley test, was conducted in a wooden alley 8 feet long and 2 feet wide. One end of the alley contained a movable tray upon which appropriate stimuli were placed. The other end of the alley contained a box for hiding. Each test began with the monkey in a start box 1 foot in front of the hiding box; thus, the animal could maintain its original position, approach the stimulus tray as it moved toward him, or flee into the hiding box. The infants were presented with five stimuli in the course of five successive days. The stimuli included a standard cloth mother, a standard wire mother, a yellow cloth mother with the head removed, a blank tray, and a large black fear stimulus, shown in Fig. 9. The infants were tested at 5, 10, and 20 days of age, respectively, and then at 20-day intervals up to 160 days. Figure 10 shows the mean number of 15-second time intervals when the infant first explored the stimulus, either by reaching for it or by stepping close to it, up to a total of 20 exposures.
Fig. 8. Change in emotionality index in fear tests.

periods spent in contact with the appropriate mother during the 90-second tests for the two single-mother groups, and the responses to the cloth mother by four infants from the dual-mother group.

During the first 80 days of testing, all the groups showed an increase in response to the respective mother surrogates. The infants fed on the single wire mother, however, reached peak responsiveness at this age and then showed a consistent decline, followed by an actual avoidance of the wire mother. During test sessions 140 to 160, only one contact was made with the wire mother, and three of the four infants ran into the hiding box almost immediately and remained there for the entire test session. On the other hand, all of the infants raised with a cloth mother, whether or not they were nursed by her, showed a progressive increase in time spent in contact with their cloth mothers until approaches and contacts during the test sessions approached maximum scores.

The development of the response of flight from the wire mother by the group fed on the single wire mother is, of course, completely contrary to a derived-drive theory of affectional development. A comparison of this group with the group raised with a cloth mother gives some support to the hypothesis that feeding or nursing facilitates the early development of responses to the mother but that without the factor of contact comfort, these positive responses are not maintained.

The differential responsiveness to the cloth mother of infants raised with both mothers, the reduced emotionality of both the groups raised with cloth mothers in the home-cage fear tests, and the development of approach responses in the straight-alley test indicate that the cloth mother provides a haven of safety and security for the frightened infant. The affectional response patterns found in the infant monkey are unlike tropistic or even complex reflex responses; they resemble instead the diverse and pervasive patterns of response to his mother exhibited by the human child in the complexity of situations involving child-mother relationships.

The role of the mother as a source of safety and security has been demonstrated experimentally for human infants by Arsenian (22). She placed children 11 to 30 months of age in a strange room containing toys and other play objects. Half of the children were accompanied into the room by a mother or a substitute mother (a familiar nursery attendant), while the other half entered the situation alone. The children in the first group (mother present) were much less emotional and participated much more fully in the play activity than those in the second group (mother absent). With repeated testing, the security score, a composite score of emotionality and play behavior, improved for the children who entered alone, but it still fell far below that for the children who were accompanied by their mothers. In subsequent tests, the children from the mother-present group were placed in the test room alone, and there was a drastic drop in the security scores. Contrariwise, the introduction of the mother raised the security scores of children in the other group.

We have performed a similar series of open-field experiments, comparing monkeys raised on mother surrogates with control monkeys raised in a wire cage containing a cheesecloth blanket from days 1 to 14 and no cloth blanket subsequently. The infants were introduced into the strange environment of the open field, which was a room measuring 6 by 6 by 6 feet, containing multiple stimuli known to elicit curiosity-manipulatory responses in baby monkeys. The infants raised with single mother surrogates were placed in this situation twice a week for 8 weeks, no mother surrogate being present during one of the weekly sessions and the appropriate mother surrogate (the kind which the experimental infant had always known) being present during the other sessions. Four infants raised with dual mother surrogates and four control infants were subjected to similar experimental sequences, the cloth mother being present on half of the occasions. The remaining four "dual-mother" infants were given repetitive tests to obtain information on the development of responsiveness to each of the dual mothers in this situation. A cloth blanket was always available as one of the stimuli throughout the sessions. It should be emphasized that the blanket could readily compete with the cloth mother as a contact stimulus, for it was standard laboratory procedure to wrap the infants in soft cloth whenever they were removed from their cages for testing, weighing, and other required laboratory activities.

As soon as they were placed in the test room, the infants raised with cloth mothers rushed to their mother surrogate when she was present and clutched her tenaciously, a response so strong that it can only be adequately depicted by motion pictures. Then, as had been observed in the fear tests in the home cage, they rapidly relaxed, showed no sign of apprehension, and began to demonstrate unequivocal positive responses of manipulating and climbing on the mother. After several sessions, the infants began to use the mother surrogate as a base of
operations, leaving her to explore and handle a stimulus and then returning to her before going to a new plaything. Some of the infants even brought the stimuli to the mother, as shown in Fig. 11. The behavior of these infants changed radically in the absence of the mother. Emotional indices such as vocalization, crouching, rocking, and sucking increased sharply. Typical response patterns were either freezing in a crouched position, as illustrated in Fig. 12, or running around the room on the hind feet, clutching themselves with their arms. Though no quantitative evidence is available, contact and manipulation of objects was frantic and of short duration, as opposed to the playful type of manipulation observed when the mother was present.

In the presence of the mother, the behavior of the infants raised with single wire mothers was both quantitatively and qualitatively different from that of the infants raised with cloth mothers. Not only did these infants spend little or no time contacting their mother surrogates but the presence of the mother did not reduce their emotionality. These differences are evident in the mean number of time periods spent in contact with the respective mothers, as shown in Fig. 13, and the composite emotional index for the two stimulus conditions depicted in Fig. 14. Although the infants raised with dual mothers spent considerably more time in contact with the cloth mother than did the infants raised with single cloth mothers, their emotional reactions to the presence and absence of the mother were highly similar, the composite emotional index being reduced by almost half when the mother was in the test situation. The infants raised with wire mothers were highly emotional under both conditions and actually showed a slight, though nonsignificant, increase in emotionality when the mother was present. Although some of the infants reared by a wire mother did contact her, their behavior was similar to that observed in the home-cage fear tests. They did not clutch and cling to their mother as did the infants with cloth mothers; instead, they sat on her lap and clutched themselves, or held their heads and bodies in their arms and engaged in convulsive jerking and rocking movements similar to the autistic behavior of deprived and institutionalized human children. The lack of exploratory and manipulatory behavior on the part of the infants reared with wire mothers, both in the presence and absence of the wire mother, was similar to that observed in the mother-absent condition for the infants raised with the cloth mothers, and such contact with objects as was made was of short duration and of an erratic and frantic nature. None of the infants raised with single wire mothers displayed the persistent and aggressive play behavior that was typical of many of the infants that were raised with cloth mothers.

The four control infants, raised without a mother surrogate, had approximately the same emotionality scores when the mother was absent that the other infants had in the same condition, but the control subjects' emotionality scores were significantly higher in the presence of the mother surrogate than in her absence. This result is not surprising, since recent evidence indicates that the cloth mother with the highly ornamental face is an effective fear stimulus.

Fig. 11. Subsequent response to cloth mother and stimulus in the open-field test.

Fig. 12. Response in the open-field test in the absence of the mother surrogate.
Fig. 13 (left). Responsiveness to mother surrogates in the open-field test. Fig. 14 (right). Emotionality index in testing with and without the mother surrogates.

for monkeys that have not been raised with her.

Further illustration of differential responsivity to the two mother surrogates is found in the results of a series of developmental tests in the open-field situation, given to the remaining four "dual-mother" infants. These infants were placed in the test room with the cloth mother, the wire mother, and no mother present on successive occasions at various age levels. Figure 15 shows the mean number of time periods spent in contact with the respective mothers for two trials at each age level, and Fig. 16 reveals the composite emotion scores for the three stimulus conditions during these same tests. The differential responsivity to the cloth and wire mothers, as measured by contact time, is evident by 20 days of age, and this systematic difference continues throughout 140 days of age. Only small differences in emotionality under the various conditions are evident during the first 85 days of age, although the presence of the cloth mother does result in slightly lower scores from the 45th day onward. However, at 105 and 145 days of age there is a considerable difference for the three conditions, the emotionality scores for the wire-mother and blank conditions showing a sharp increase. The heightened emotionality found under the wire-mother condition was mainly contributed by the two infants fed on the wire mother. The behavior of these two infants in the presence of the wire mother was similar to the behavior of the animals raised with a single wire mother. On the few occasions when contact with the wire mother was made, the infants did not attempt to cling to her; instead they would sit on her lap, clasp their heads and bodies, and rock back and forth.

In 1953 Butler (23) demonstrated that mature monkeys enclosed in a
A dimly lighted box would open and reopen a door for hours on end with no other motivation than that of looking outside the box. He also demonstrated that rhesus monkeys showed selectivity in rate and frequency of door-opening in response to stimuli of different degrees of attractiveness (24). We have utilized this characteristic of response selectivity on the part of the monkey to measure the strength of affectional responsiveness of the babies raised with mother surrogates in an infant version of the Butler box. The test sequence involves four repetitions of a test battery in which the four stimuli of cloth mother, wire mother, infant monkey, and empty box are presented for a 30-minute period on successive days. The first four subjects raised with the dual mother surrogates and the eight infants raised with single mother surrogates were given a test sequence at 40 to 50 days of age, depending upon the availability of the apparatus. The data obtained from the three experimental groups and a comparable control group are presented in Fig. 17. Both groups of infants raised with cloth mothers showed approximately equal responsiveness to the cloth mother and to another infant monkey, and no greater responsiveness to the wire mother than to an empty box. Again, the results are independent of the kind of mother that lactated, cloth or wire. The infants raised with only a wire mother and those in the control group were more highly responsive to the monkey than to either of the mother surrogates. Furthermore, the former group showed a higher frequency of response to the empty box than to the wire mother.

In summary, the experimental analysis of the development of the infant monkey's attachment to an inanimate mother surrogate demonstrates the overwhelming importance of the variable of soft body contact that characterized the cloth mother, and this held true for the appearance, development, and maintenance of the infant-surrogate-mother tie. The results also indicate that, without the factor of contact comfort, only a weak attachment, if any, is formed. Finally, probably the most surprising finding is that nursing or feeding played either no role or a subordinate role in the development of affection as measured by contact time, responsiveness to fear, responsiveness to strangeness, and motivation to seek and see. No evidence was found indicating that nursing mediated the development of any of these responses, although there is evidence indicating that feeding probably facilitated the early appearance and increased the early strength of affectional responsiveness. Certainly feeding, in contrast to contact comfort, is neither a necessary nor a sufficient condition for affectional development.

Retention of Affectional Responses

One of the outstanding characteristics of the infant's attachment to its mother is the persistence of the relationship over a period of years, even though the frequency of contact between infant and mother is reduced with increasing age. In order to test the persistence of the responsiveness of our "mother-surrogate" infants, the first four infant monkeys raised with dual mothers and all of the monkeys raised with single mothers were separated from their surrogates at 165 to 170 days of age. They were tested for affectional retention during the following 9 days, then at 30-day intervals during the following year. The results are of necessity incomplete, inasmuch as the entire mother-surrogate program was initiated less than 2 years ago, but enough evidence is available to indicate that the attachment formed to the cloth mother during the first 6 months of life is enduring and not easily forgotten.

Affectional retention as measured by the modified Butler box for the first 15 months of testing for four of the infants raised with two mothers is given in Fig. 18. Although there is considerable variability in the total response frequency from session to session, there is a consistent difference in the number of responses to the cloth mother as contrasted with responses to either the wire mother or the empty box, and there is no consistent difference between responses to the wire mother and to the empty box. The effects of contact comfort versus feeding are dramatically demonstrated in this test by the monkeys raised with either single cloth or wire mothers. Figure 19 shows the frequency of response to the appropriate mother surrogate and to the blank box during the preexposure period and the first 90 days of retention testing. Removal of the mother resulted in a doubling of the frequency of response to the cloth mother and more than tripled the difference between the responses to the cloth mother and those to the empty box for the infants that had lived with a single nonlactating cloth mother surrogate. The infants raised with a single lactating wire mother, on the other hand, not only failed to show any consistent preference for the wire mother but also showed a highly significant reduction in general level of responding. Although incomplete, the data from further retention testing indicate that the difference between these two groups persists for at least 5 months.

Affectional retention was also tested
in the open field during the first 9 days after separation and then at 30-day intervals. Each test condition was run twice in each retention period. In the initial retention tests the behavior of the infants that had lived with cloth mothers differed slightly from that observed during the period preceding separation. These infants tended to spend more time in contact with the mother and less time exploring and manipulating the objects in the room. The behavior of the infants raised with single wire mothers, on the other hand, changed radically during the first retention sessions, and responses to the mother surrogate dropped almost to zero. Objective evidence for these differences are given in Fig. 20, which reveals the mean number of time periods spent in contact with the respective mothers. During the first retention test session, the infants raised with a single wire mother showed almost no responses to the mother surrogate they had always known. Since the infants raised with both mothers were already approaching the maximum score in this measure, there was little room for improvement. The infants raised with a single nonlactating cloth mother, however, showed a consistent and significant increase in this measure during the first 90 days of retention. Evidence for the persistence of this responsiveness is given by the fact that after 15 months' separation from their mothers, the infants that had lived with cloth mothers spent an average of 8.75 out of 12 possible time periods in contact with the cloth mother during the test. The incomplete data for retention testing of the infants raised with only a lactating wire mother or a nonlactating cloth mother indicates that there is little or no change in the initial differences found between these two groups in this test over a period of 5 months. In the absence of the mother, the behavior of the infants raised with cloth mothers was similar in the initial retention tests to that during the preseparation tests, but with repeated testing they tended to show gradual adaptation to the open-field situation and, consequently, a reduction in their emotionality scores. Even with this over-all reduction in emotionality, these infants had consistently lower emotionality scores when the mother was present.

At the time of initiating the retention tests, an additional condition was introduced into the open-field test: the surrogate mother was placed in the center of the room and covered with a clear Plexiglas box. The animals raised with cloth mothers were initially disturbed and frustrated when their efforts to secure and contact the mother were blocked by the box. However, after several violent crashes into the plastic, the animals adapted to the situation and soon used the box as a place of orientation for exploratory and play behavior.

In fact, several infants were much more active under these conditions than they were when the mother was available for direct contact. A comparison of the composite emotionality index of the babies raised with a single cloth or wire mother under the three conditions of no mother, surrogate mother, and surrogate-mother-box is presented in Fig. 21. The infants raised with a single cloth mother were consistently less emotional when they could contact the mother but also showed the effects of her visual presence, as their emotionality scores in the plastic box condition were definitely lower than their scores when the mother was absent. It appears that the infants gained considerable emotional security from the presence of the mother even though contact was denied.

In contrast, the animals raised with only lactating wire mothers did not show any significant or consistent trends during these retention sessions other than a general over-all reduction of emotionality, which may be attributed to a general adaptation, the result of repeated testing.

Affectional retention has also been measured in the straight-alley test mentioned earlier. During the preseparation tests it was found that the infants that had only wire mothers developed a general avoidance response to all of the stimuli in this test when they were about 100 days of age and made few, if any, responses to the wire mother during the final test sessions. In contrast, all the infants raised with a cloth mother responded positively to her. Maternal separation did not significantly change the behavior of any of the groups. The babies raised with just wire mothers continued to flee into the hiding booth in the presence of the wire mother, while all of the infants raised with
cloth mothers continued to respond positively to the cloth mother at approximately the same level as in the preexposure tests. The mean number of time periods spent in contact with the appropriate mother surrogates for the first 3 months of retention testing are given in Fig. 2. There is little, if any, waning of responsiveness to the cloth mother during these 3 months. There appeared to be some loss of responsiveness to the cloth mother in this situation after 5 to 6 months of separation, but the test was discontinued at that time as the infants had outgrown the apparatus.

The retention data from these multiple tests demonstrate clearly the importance of body contact for the future maintenance of affectional responses. Whereas several of the measures in the preexposure period suggested that the infants raised with only a wire mother might have developed a weak attachment to her, all responsiveness disappeared in the first few days after the mother was withdrawn from the living cage. Infants that had had the opportunity of living with a cloth mother showed the opposite effect and either became more responsive to the cloth mother or continued to respond to her at the same level.

These data indicate that once an affectional bond is formed it is maintained for a very considerable length of time with little reinforcement of the contact-comfort variable. The limited data available for infants that have been separated from their mother surrogates for a year suggest that these affectional responses show resistance to extinction similar to the resistance previously demonstrated for learned fears and learned pain. Such data are in keeping with common observation of human behavior.

It is true, however, that the infants raised with cloth mothers exhibit some absolute decrease in responsiveness with time in all of our major test situations. Such results would be obtained even if there were no true decrease in the strength of the affectional bond, because of familiarization and adaptation resulting from repeated testing. Therefore, at the end of 1 year of retention testing, new tests were introduced into the experimental program.

Our first new test was a modification of the open-field situation, in which basic principles of the home-cage fear test were incorporated. This particular choice was made partly because the latter test had to be discontinued when the mother surrogates were removed from the home cages.

For the new experiment a Masonite floor marked off in 6- by 12-inch rectangles was placed in the open-field chamber. Both mother surrogates were placed in the test room opposite a plastic start-box. Three fear stimuli, selected to produce differing degrees of emotionality, were placed in the center of the room directly in front of the start-box in successive test sessions. Eight trials were run under each stimulus condition, and in half of the trials the most direct path to the cloth mother was blocked by a large Plexiglas screen, illustrated in Fig. 23. Thus, in these trials the infants were forced to approach and bypass the fear stimulus or the wire mother, or both, in order to reach the cloth mother. Following these 24 trials with the mothers present, one trial of each condition with both mothers absent was run, and this in turn was followed by two trials run under the most emotion-provoking condition: with a mechanical toy present and the direct path to the mother blocked.

We now have complete data for the first four infants raised with both a cloth and a wire mother. Even with this scanty information, the results are obvious. As would be predicted from our other measures, the emotionality scores for the three stimuli were significantly different and these same scores were increased greatly when the direct path to the mother was blocked. A highly significant preference was shown for the cloth mother under both conditions (direct and blocked path), although the presence of the block did increase the number of first responses to the wire mother from 3 to 10 percent. In all cases this was a transient response and the infants subsequently ran on to the cloth mother and clung tightly to her. Objective evidence for this overwhelming preference is indicated in Fig. 24, which shows the mean number of time periods spent in contact with the two mothers. After a number of trials, the infants would go first to the cloth mother and then, and only then, would go out to explore, manipulate, and even attack and destroy the fear stimuli. It was as if they believed that their mother would protect them, even at the cost of her life—little enough to ask in view of her condition.

The removal of the mother surrogates from the situation produced the predictable effect of doubling the emotionality index. In the absence of the mothers, the infants would often run to the Plexiglas partition which formerly had blocked their path to the mother, or they would crouch in the corner behind the block where the mother normally would have been. The return of the mothers in the final two trials of the test in which the most emotion-evoking situation was presented resulted in behavior near the normal level, as measured by the emotionality index and contacts with the cloth mother.

Our second test of this series was designed to replace the straight-alley test described above and provide more quantifiable data on responsiveness to fear stimuli. The test was conducted in an alley 8 feet long and 2 feet wide.

![Fig. 23. Typical response to cloth mother in the modified open-field test.](image-url)
At one end of the alley and directly behind the monkeys' restraining chamber was a small stimulus chamber which contained a fear object. Each trial was initiated by raising an opaque sliding door which exposed the fear stimulus. Beginning at a point 18 inches from the restraining chamber, the alley was divided lengthwise by a partition; this provided the infant with the choice of entering one of two alleys.

The effects of all mother combinations were measured; these combinations included no mothers, two cloth mothers, two wire mothers, and a cloth and a wire mother. All mother conditions were counterbalanced by two distance conditions—distances of 24 and 78 inches, respectively, from the restraining chamber. This made it possible, for example, to provide the infant with the alternative of running to the cloth mother which was in close proximity to the fear stimulus or to the wire mother (or no mother) at a greater distance from the fear stimulus. Thus, it was possible to distinguish between running to the mother surrogate as an object of security, and generalized flight in response to a fear stimulus.

Again, the data available at this time are from the first four infants raised with cloth and wire mothers. Nevertheless, the evidence is quite conclusive. A highly significant preference is shown for the cloth mother as compared to the wire mother or to no mother, and this preference appears to be independent of the proximity of the mother to the fear stimulus. In the condition in which two cloth mothers are present, one 24 inches from the fear stimulus and the other 78 inches from it, there was a preference for the nearest mother, but the differences were not statistically significant. In two conditions in which no cloth mother was present and the infant had to choose between a wire mother and no mother, or between two empty chambers, the emotionality scores were almost twice those under the cloth-mother-present condition.

No differences were found in either of these tests that were related to previous conditions of feeding—that is, to whether the monkey had nursed on the cloth or on the wire mother.

The results of these two new tests, introduced after a full year's separation of mother surrogate and infant, are comparable to the results obtained during the preseparation period and the early retention testing. Preferential responses still favored the cloth as compared to the wire mother by as much as 85 to 90 percent, and the emotionality scores showed the typical 2:1 differential ratio with respect to mother-absent and mother-present conditions.

The research presented here on the analysis of two affectional variables through the use of objective and observational techniques suggest a broad new field for the study of emotional development of infant animals. The analogous situations and results found in observations and study of human infants and of subprimes demonstrate the apparent face validity of our tests. The reliability of our observational techniques is indicated, for example, by the correlation coefficients computed for the composite emotional index in the open-field test. Four product-moment correlation coefficients, computed from four samples of 100 observations by five different pairs of independent observers over a period of more than a year, ranged from .87 to .89.

Additional Variables

Although the overwhelming importance of the contact variable has been clearly demonstrated in these experiments, there is reason to believe that other factors may contribute to the development of the affectional response pattern. We are currently conducting a series of new experiments to test some of these postulated variables.

For example, Bowlby (14) has suggested that one of the basic affectional variables in the primate order is not just contact but clinging contact. To test this hypothesis, four infant monkeys are being raised with the standard cloth mother and a flat inclined plane, tightly covered with the same type of cloth. Thus, both objects contain the variable of contact with the soft cloth, but the shape of the mother tends to maximize the clinging variable, while the broad flat shape of the plane tends to minimize it. The preliminary results for differences in responsiveness to the cloth mother and responsiveness to the inclined plane under conditions that produce stress or fear or visual exploration suggest that clinging as well as contact is an affectional variable of considerable importance.

Experiments now in progress on the role of rocking motion in the development of attachment indicate that this may be a variable of measurable importance. One group of infants is being raised on rocking and stationary mothers and a second group, on rocking and stationary inclined planes. Both groups of infants show a small but consistent preference for the rocking object, as measured in average hours spent on the two objects.

Preliminary results for these three groups in the open-field test give additional evidence concerning the variable of clinging comfort. These data revealed that the infants raised with the standard cloth mother were more responsive to their mothers than the infants raised with inclined planes were to the planes.

The discovery of three variables of measurable importance to the formation and retention of affection is not surprising, and it is reasonable to assume that others will be demonstrated. The data so far obtained experimentally are in excellent concordance with the affectional variables named by Bowlby (14). We are now planning a series of studies to assess the effects of consistency and inconsistency with respect to the mother surrogates in relation to the clinical concept of rejection. The effects of early, intermediate, and late maternal deprivation and the generalization of the infant-surgeon attachment in social development are also being investigated. Indeed, the strength and stability of the monkeys' affectional responses to a mother surrogate are such that it should be practical to determine the neurological and biochemical variables that underlie love.

References and Notes

5. J. Jaynes, ibid., in press.
Amino Acid Metabolism in Mammalian Cell Cultures

Harry Eagle

A number of cell lines derived from normal and malignant tissues have now been serially propagated in culture. These may be grown adherent to a glass surface and overlaid with a fluid medium, or they may be grown in suspension; but in either case the opportunity is presented for the study of metabolism at the cellular level in a system which operationally resembles bacterial cultures in most essential respects. The cells and the medium can be separately analyzed, balance experiments can be set up, metabolic processes can be examined qualitatively and quantitatively under controlled conditions, and the corresponding enzymatic activities can be explored in cell-free extracts.

A relatively limited number of metabolites have been shown to suffice for the apparently indefinite propagation of all the human cell lines so far studied. The minimal medium, in which every component is demonstrably essential, is listed in Table 1 and includes 29 components: 13 amino acids, 8 vitamins, 6 ionic species, glucose, and serum protein (1, 2). The role of the serum protein is not entirely clear. Although a few cell lines have been serially propagated in a protein-free medium (3), serum protein is required by most mammalian cell cultures. In monolayer cultures, the protein plays a role in the adhesion of the cells to glass (4). However, its essentiality in suspension cultures indicates that this is not its only function, and it seems reasonable to assume that it acts in part as a carrier of as yet unidentified growth factors which are bound to the protein, and which are slowly released into the medium.

The present article, dealing with the amino acid metabolism of these cultured cells, is a progress report rather than a review and in large part summarizes studies from a single laboratory. The enormous body of information available with respect to amino acid metabolism in bacterial cultures (5, 6) has served as a stimulus and prototype for the studies here reported. The relevant findings in bacterial systems have not been referred to in detail, only because that important exercise in comparative biochemistry is beyond the scope of the present report.

Nutritionally Essential Amino Acids

The classic experiments of Rose and his coworkers (7) have shown that eight amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) suffice for nitrogen balance in feeding experiments in man. In contrast, every cell culture so far examined, whether human or animal (8) in origin, and whether deriving from normal or malignant tissue (9), has required at least 13 amino acids for survival and growth. Over and above the eight amino acids required for nitrogen balance, these cell cultures require arginine, cyst(e)ine, glutamine, histidine, and tyrosine, and on the omission of any one of these, the cells degenerate and die. In their early stages these degenerative processes are reversible. This presents an opportunity to explore the intimate structural derangement caused by specific amino acid deficiencies, and the reparative processes which occur on restoration of these amino acids to the medium.

None of the L-amino acids substitute for the L-isomer (8) except for cyst(e)ine, and this apparently acts by mobilizing cyst(e)ine residues bound to the serum protein of the medium. (This is discussed below.) Dipeptides were found to be active, substituting for both component amino acids (10). Recent experiments indicate that, although dipeptides are hydrolyzed extracellularly by serum peptides, and perhaps by cell-derived peptidases, some of the dipeptide is transported into the cell and there hydrolyzed.

A number of keto acids and other amino acid congeners have been tested with respect to their ability to substitute for the corresponding amino acid (11). The results with several human and animal cell lines are summarized in Table 2. The cells contain a wide variety of transaminases (12), and, as determined in feeding experiments in rats (5), most of these keto acids do in fact substitute for the corresponding amino acid.

Of particular interest is the fact that, with all the human cell lines studied, citrulline substituted for arginine, while ornithine was inactive [Table 2; see also (13)]. Attempts to encourage citrulline biosynthesis by progressive removal of

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