Table 1. Mean simple auditory reaction times according to the phase of the cardiac cycle in which the stimuli were presented; 31 men, 25 women.*

| Item | Time (sec) | | | |
|---|---|---|---|---|
| | P | QRS | T | T-P |
| | | *Men* | | |
| Mean | 0.1418 | 0.1502 | 0.1487 | 0.1481 |
| S.D. | .023 | .0276 | .035 | .0275 |
| | | *Women* | | |
| Mean | .1482 | .1568 | .1574 | .1546 |
| S.D. | .0199 | .0283 | .0287 | .0295 |

* Mixed-model analyses of variance were carried out separately for males and females and for the combined groups; the Hotelling $T^2$ statistics were significant at nearly the .10 level in each of the separate analyses (*9*) and at the .01 level for the combined sexes. The differences in mean reaction times to stimuli presented at QRS and P phases were tested by the Scheffe Multiple Comparison Procedure (*9*); significance obtained at the .05 level for males and at the .10 level for females, and at the .005 level in the combined analysis (*10*).

the arterial system (*5*), in turn affect the central nervous system and can thus influence not only the quantity of electrical activity in the efferent cardiovascular nerves—within the time span of a heart cycle (*6*)—but also the frequency and amplitude of the electrocorticogram (*7*). There also may be an association between the number of erroneous psychomotor responses and the phase of the cardiac cycle in which the stimuli are presented (*8*). These facts suggest that it is desirable to determine whether there is a relationship between simple reaction time and the cardiac cycle. Specifically, the speed of simple auditory reaction time was studied in relation to the phases of the cardiac cycle in which the stimuli occurred.

The experimental procedure required the subject to react as quickly as he could to an auditory signal by lifting his index finger from a telegraph key.

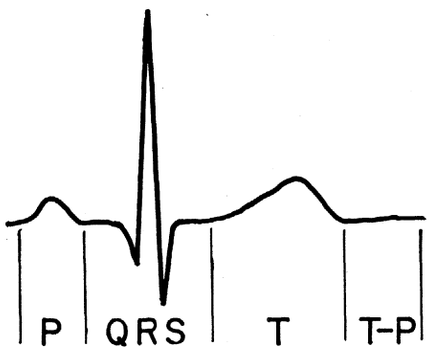


Fig. 1. Representation of the cardiac cycle by a normal electrocardiogram. Vertical lines arbitrarily divide the cycle into the four intervals used for the analysis of the reaction time data as shown in Table 1. Letters are those conventionally used to designate the principal deflections of the EKG.

A simultaneous electrocardiograph record was marked with a signal for the occurrence of the stimulus. Reaction times were read from a time clock which recorded to 0.01 second. After instructions and example stimuli, each subject had 100 reaction-time measurements. The auditory stimulus was a 1000-cycle tone presented through earphones at about 80 db. After each reaction the subject waited for 2 seconds after which a warning lamp glowed and the subject replaced his finger on the key. One second after he replaced his finger, the auditory signal was presented. As "catch stimuli" 10-second delays (10 percent) were included at random in the series in place of the regular 1-second intervals. Reactions to catch stimuli and the succeeding reaction times were not included in the analyses of the data. The reaction times of each subject were categorized according to four phases of the cardiac cycle in which the corresponding stimuli were presented. For each subject, a median reaction time was derived for each of the four phases of the electrocardiogram (EKG). A total of 31 men and 25 women between the ages of 20 and 30 years, were the subjects, of whom 9 were normal volunteers of the Clinical Center of the National Institutes of Health and 47 were employees.

Figure 1 shows the intervals into which the EKG was divided in the analysis. Mean reaction times corresponding to these intervals are given in Table 1.

The data show reactions to stimuli presented during the P-phase of the EKG were significantly faster than to stimuli presented during subsequent intervals. The difference between the P and QRS interval reaction times was 0.0094 second for the men and 0.0086 second for the women. These differences are about the same order of magnitude as previously reported for variations in reaction time as a function of the EEG (*4*). Apparently cyclical changes in alerting are associated with both the EEG and the EKG. Although the results clearly show a significant difference ($p < .01$) in favor of a faster reaction time when the stimuli are presented during the P-phase of the EKG, not all subjects displayed the phenomenon. This may be a matter of rather basic individual differences or possibly a matter of the degree of physiological arousal at the time the subjects were measured. A number of physiological mechanisms might account for the present results. Further studies are needed to identify mechanisms in this relationship. This variation in reaction time should not be confused with a relationship of reaction time and vascular disease which, because of damage to the nervous system, may result in a slowing of reaction time.

JAMES E. BIRREN
PHILIPPE V. CARDON, JR.
SHIRLEY L. PHILLIPS
*National Institute of Mental Health, Bethesda 14, Maryland*

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7 February 1963

## Inheritance of Behavior in Infants

Abstract. *Mental and motor abilities and personality development were studied in 20 pairs of infant twins of the same sex on a monthly basis in their first year, that is, before mutual imitation becomes a factor. Blood group determinations, made after the study was completed, revealed an* $N_1$ *of 11 fraternal and* $N_2$ *of 9 identical pairs. Within-pair differences were significantly greater within fraternal pairs on all tests and rating scales.*

The majority of longitudinal studies of infants and children have indicated that there is consistency in personality within individuals over the years (*1*), but the role that heredity has played in this can only be surmised. We have applied the twin method to a longitudinal study in order to investigate the role of heredity.

Twenty pairs of twins of the same sex were examined on a monthly basis in their own homes in their first year. Zygosity was determined at the end of the study on the basis of non-concordance or concordance on 13 blood-group factors. We found an $N_1$ of 11 fraternal

pairs and an $N_2$ of 9 identical pairs. All families entered the study voluntarily, apparently because of an interest in gauging development of their twins. None was paid. Most were middle class and represented a variety of racial and cultural backgrounds. The tests included the Mental and Motor Scales and the Infant Behavior Profile developed by Nancy Bayley of the National Institute of Mental Health. Our report is based on the scores of these instruments (*2*).

The present approach has several advantages: (i) According to Piaget, deferred imitation of other children, that is, imitation independent of immediate perception, starts after the first year (*3*). Such imitation was not observed in our group. Our observations also bear out Ahrens' findings that infants have little interest in one another before the 10th month, after which interest gradually increases (*4*). Thus, mutual imitation and "contagion" within pairs can be ruled out as factors in our results. (ii) Differential treatment of identical and fraternal twins by parents can also be effectively ruled out as a contaminating factor. Neither examiners nor parents were certain of zygosity, since determinations were made only at the end of the study. Parents who ventured an opinion tended to believe their twins were fraternal; hence parents of fraternals were correct, and parents of identicals were incorrect in six out of the nine pairs. Obstetricians were of little help in determining zygosity, for they were incorrect 9 out of 19 times. (Our observations and careful discussions with parents indicated that, as a rule, differential behavior of infants drew different rather than the same responses from parents. In no case did a parent "create" differences where none previously existed.) (iii) Two investigators worked independently, each seeing approximately half the twin pairs. Their data formed similar distributions on all measures used and suggests that the results are readily reproducible (Figs. 1–3).

Figure 1 shows the distribution of intra-pair differences on the combined Mental and Motor Scales, and it is clear that identical pairs and fraternal pairs form two distinct but overlapping populations. [$P < .01$; all $P$ values are based on one-tailed tables of the Mann-Whitney nonparametric test (*5*).] The Mental Scale ($P < .10$) and the Motor Scale ($P < .005$) follow a similar order when plotted individually. Figure 2 illustrates the distribution of intrapair differences on the Infant Behavior Profile, where once again fraternal pairs exhibit greater differences ($P < .001$).

The extent of within-pair consistency over the first year is indicated by the

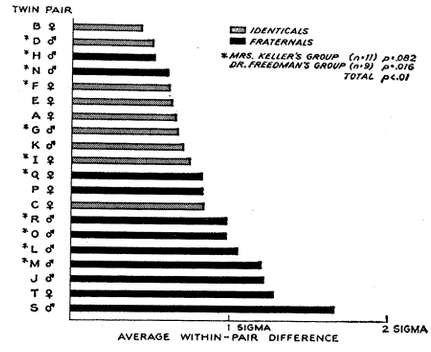


Fig. 1. The Bayley Mental and Motor Scales averaged to form a single distribution (*2*). Average within-pair differences in the first year, based on 8 to 12 monthly administrations.

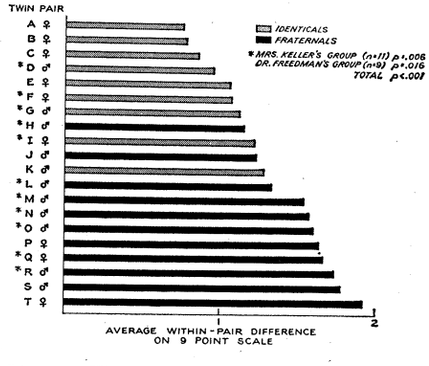


Fig. 2. The Bayley Infant Behavior Profile, a rating scale consisting of 21 items covering 12 categories of behavior (*2*). Average within-pair differences in the first year, based on 8 to 12 monthly administrations.

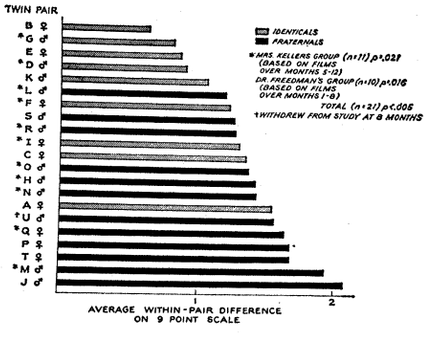


Fig. 3. Within-pair differences on the Bayley Infant Behavior Profile, based on 8 consecutive months of filmed behavior (either months 1 to 8, or months 5 to 12).

following: On the Mental Scales, in identical twins within-pair changes in superiority occurred in 37 percent of the tests administered; fraternal twins switched positions on 23 percent of consecutive tests. Likewise, on the Motor Scales, identical pairs switched positions on 35 percent of the tests compared to 15 percent in fraternal pairs. Ranking indicated that fraternal pairs exhibited significantly fewer changes vis-à-vis each other on both the Mental Scales ($P = .05$) and the Motor Scales ($P = .005$).

Similar results were obtained on the 21-item Infant Behavior Profile (*2*). Fraternal pairs averaged 4.36 items on which no more than one switchover in relative position occurred over the first year, and on which there was an average within-pair difference of two or more points, that is, items in which intrapair differences were decidedly persistent and large; identical twins averaged 1.12. Ranking on this basis again differentiated identical from fraternal twins ($P < .025$). Items rated on the basis of motor activity most often met the above criteria although other categories of behavior proved equally discriminating in particular pairs.

Judgment of behavior from films is perhaps the best-controlled aspect of this study. Monthly motion pictures were taken in which each twin of a pair was filmed separately in the same situations. At the end of the study, the films of one twin were shown to a group of four professionals who had worked with infants, and the films of the other were shown to a second comparable group. In this way we avoided a possible "halo" effect. The judges rated each child on the Infant Behavior Profile, and the scores were averaged for each infant. The difference within each pair was ascertained, the differences ranked, and again intrapair differences among fraternal twins were distinctly larger ($P < .005$) (Fig. 3). The rank-order correlation between the distributions in Figs. 2 and 3 is .44 ($P < .03$).

The distribution of within-pair differences on the Infant Behavior Profile (Fig. 2) reflects our experiences in recording data after visits to the homes of identical pairs A, B, C, and D. In each of these pairs the personalities merged into a single picture after a few hours, and unless our impressions of differences were recorded immediately, it became impossible to do so later.

This merger could not be ascribed to similar appearance, for there was no difficulty in recording other identical looking pairs who exhibited some clear-cut behavioral differences.

Consistent behavioral differences within some identical pairs deserves special attention. In pair E, following normal births, only the second-born was startled by noises in the first two months, cried at the jack-in-the-box at 3 months, and became extremely fearful of strangers during the last half of the first year. In pair K, the second-born twin, after a traumatic breech birth, slept much of the time over the first 1½ months. Then he began smiling to people more readily than did his brother whose delivery had been normal, and at 5 months he wanted to be picked up by any newcomer. Thereafter he remained more immediately outgoing to people than his twin. Our observations and interviews with the parents suggested that differential treatment played no role in producing these differences, and obstetrical and pediatric records yielded no clues. The following categories were examined, and none could be reasonably associated with such differences: birth order, traumatic delivery, large differences in birth weight, Apgar ratings (an assessment of viability at birth), and monochorionic versus dichorionic embryogenesis.

Although motor behavior was most effective in differentiating within twin-pairs, perhaps because it was most objectively scored, we cannot estimate heritability for specific aspects of behavior, nor was this our aim. Our question was rather: Does heredity, in a general sense, play a role in the development of abilities and personality? An affirmative answer would appear to be warranted (*6*).

D. G. FREEDMAN*
BARBARA KELLER

*Langley Porter*
*Neuropsychiatric Institute,*
*San Francisco 22, California*

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* Present address: Department of Psychology, University of California, Davis.

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## Cholesterol in Higher Plants

Abstract. *The presence of cholesterol in* Solanum tuberosum *and* Dioscorea spiculiflora *plants was demonstrated by gas-liquid chromatography, thin-layer chromatography, isolation and mixed melting point, and purification to constant radioactivity after dilution with authentic cholesterol.*

Although cholesterol, the principal animal sterol, has been isolated from red algae (*1*), its presence in higher plants has not previously been reported. In the course of our work on the biosynthesis of steroids in plants (*2*) we have identified this sterol in the sterol fraction of both *Solanum tuberosum* and *Dioscorea spiculiflora*.

Four young potato plants, *Solanum tuberosum*, of the Katahdin variety (*3*), were fed 25 $\mu$c each of mevalonic acid-2-$C^{14}$ by the wick method (*4*) and allowed to grow for 1 month. The stems and leaves were homogenized with water in a Waring blender, hydrolyzed with 4*N* HCl, and filtered; the filter cake was washed with water to neutrality. This residue was extracted with light petroleum in a Soxhlet apparatus.

The extract (4.987 g) was chromatographed on neutral alumina (grade III), and the fraction was eluted with 50 percent benzene-light petroleum and again chromatographed. This gave 213 mg of crude sterols, which were acetylated and chromatographed on alumina. Gas-liquid chromatography (*5*) and thin-layer chromatography (TLC) on Anasil B (*6*), developed four times with a mixture of hexane and ether (96:4) indicated that this fraction contained a mixture of three sterol acetates with properties similar to the authentic acetates of stigmasterol, $\beta$-sitosterol, and cholesterol. A 53-mg sample of this mixture was chromatographed on 30-g columns of Anasil S by gradient elution (1 liter of 1 percent ether in hexane with increasing amounts of 20 percent ether in hexane) (*7*). Partial resolution of this mixture was obtained in the first 300 ml, which was rechromatographed. The middle fractions were enriched in a component with the mobility of cholesterol acetate, and the mixture was further purified by preparative TLC. Finally, a fraction was obtained which gave a single spot in thin-layer chromatograms on Anasil B. This fraction had the same mobility as an authentic sample of cholesterol acetate.

Gas-liquid chromatography of this fraction showed it to be a 1:1 mixture of two compounds corresponding in their retention times to cholesterol acetate and sitosterol acetate, respectively, although the latter had been completely removed by preparative TLC. When attempts to resolve the mixture by TLC failed, 2.2 mg of this mixture were subjected to preparative gas-liquid chromatography (*8*).

The fraction with the retention time of cholesterol acetate (200 $\mu$g) was recrystallized from 0.1 ml of methyl alcohol at 5°C and gave crystals melting at 112° to 116.5°C. The melting point of a mixture of this material with pure cholesterol acetate (mp 116.5° to 117.5°) was 114° to 117°C.

The crystals were then combined with the mother liquor, diluted with 26.3 mg of cholesterol acetate, and hydrolyzed to cholesterol. This material, having a radioactivity of 11,400 count/min per millimole, was purified through the dibromide (*9*). The radioactivity dropped to 8940 count/min per millimole and was not further changed by repetition of the dibromide purification step or by recrystallization from a mixture of ethanol and water.

Six young *Dioscorea spiculiflora* plants were fed 25 $\mu$c each of mevalonic acid-2-$C^{14}$ through the stems for 30 days by the wick technique. The tubers and the upper portions of the plants were worked up separately as follows. After homogenization with water, the insoluble material was filtered off and extracted with petroleum ether and then with ethanol in a Soxhlet apparatus. The ethanol extract was combined with the filtrate and hydrolyzed