THE ONTOGENY OF THE CLASSICAL CONDITIONED LEG FLEXION RESPONSE IN NORMAL, DL PARA CHLOROPHENYLALANINE TREATED AND RADIOTHYROIDECTOMIZED ALBINO RATS

A Dissertation

Presented to

the Faculty of the Department of Psychology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

By

M. Marlyne Kilbey

May, 1969

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ABSTRACT

Habituation and classical conditioning were investigated during the cortical developmental period in normal and experimentally analogous phenylketonuric and cretin rats.

Three hundred and twenty Ss comprised 32 equal groups. These formed conditioning (T1, T2, T3, and T4), pseudoconditioning (T5), drug control (T6 and T7), and normal control (T8) groups at four age levels designated A1, A2, A3, and A4. The A1 Ss were tested at an average age of seven days, A2 at 14, A3 at 21, A4 at 28. The range of ages for each group was three days. Daily intubations of dl para chlorophenylalanine (dl PCL) suspended in a .20 per cent agar solution (T1, T2, T6 and T7 groups), or of the vehicle (T3, T4, T5 and T8 groups), were initiated within eight hours after birth and discontinued 72 hours prior to conditioning (T1 to T5 groups) or 24 hours prior to sacrifice (T6 to T8 groups). Intubated volume was 10 ml/kg. Groups T1 and T6 received 150 mg/kg dl PCL with A3 and A4 levels being switched to 100 mg/kg on day 13. Groups T2 received 100 mg/kg dl PCL. At birth T3 groups were injected with 150 microcuries of radioactive iodine.

Eight blocks of ten trials pairing a vibro-tactile conditioned stimulus (CS) and a shock unconditioned stimulus (UCS) and one block of only CS presentations were completed. Nonsystematic presentations of CS and UCS constituted a pseudoconditioning control. Anticipatory conditioned responses (ACR) and test conditioned responses (TCR) were recorded. Two periods of spontaneous leg flexion (SLF) were recorded to measure general activity.

Five measures were recorded which were used to equate CS and UCS intensity levels for the individual Ss. The number of trials necessary to reach an habituation criterion for CS presentation was determined.

Whole brain serotonin (5-HT) and norepinephrine (NE) and plasma phenylalanine were assayed for all Ss. Thyroid gland tissue for 14 T3 and 14 T4 Ss was histologically examined.

Unless otherwise noted, the results designated as significant refer to p less than .01. The TCRs yielded significant treatment and age effects. Normal groups (T4) evidenced significantly superior conditioning. Conditioning level was significantly higher (p less than .05) for T4 Ss at A3 (21 days) than at A1 or A2, while it did not differ from that at A4.

The ACR data showed all factors, except treatment by age by block, to be significant. Age and treatment by age effects were significant in analysis of SLFs preceding conditioning. The SLF measures during conditioning yielded significant treatment and age effects. The five measures taken to establish CS and UCS levels yielded significant main and interaction effects. Analysis of the number of trials to reach an habituation criterion for CS presentation did not show significance.

Brain NE and 5-HT data were significant for all factors. Brain 5-HT levels were significantly decreased for groups T1, T2, T6, and T7 (d1 PCL treated groups). Plasma phenylalanine data were significant for all factors. The levels were significantly higher in T6 and T7 (drug control) groups than in T1 and T2 (drug conditioning) groups which, in turn, had levels siginificantly higher than the other groups. Plasma phenylalanine

and brain 5-HT levels demonstrated normal developmental patterns for T8 groups, while NE levels did not. Histological examination of thyroid tissue showed T3 Ss to be athyrotic.

Comparison on all factors were significant for weight data. Groups T1, T2, and T3 weighed significantly less than T4 and T5 with the differences increasing with age.

The superior performance of T4 over T5 groups was interpreted as indicating that learning was measured by the experimental paradigm. The significant increase in TCRs obtained at 21 and 28 days indicated that conditionability increased with age during the cortical developmental period.

The other data were interpreted as evidence that experimental analogs of phenylketonuria and cretinism were established and that these conditions were incompatible with the acquisition of conditioned responses. Biochemical data which support this interpretation showed 5-HT levels of the dl PCL treated groups to be significantly lower than those of the other groups and plasma phenylalanine levels to be significantly higher. Supporting anthropometric data showed T1, T2, and T3 groups to weigh less than other groups. Supporting behavioral data showed T1, T2, and T3 groups, at all ages, to manifest lower conditioning levels than normal Ss. Supporting histological evidence showed an absence of viable thyroid tissue in T3 Ss.

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CHAPTER 1

INTRODUCTION

Developmental psychopharmacology may be defined as that area of investigation which seeks to establish the consequences of biochemical intervention on developing behavioral systems. Work in this area was reviewed by Young (1967) who remarked that the surprisingly few existing studies are extremely provocative insofar as they suggest that our understanding of physiological and developmental mechanisms underlying behavior may be considerably enlarged by additional investigations of this type.

One research goal of developmental psychopharmacology is the establishment of appropriate experimental analogs of human conditions of mental retardation for which physiological and/or biochemical correlates can be demonstrated in order that the relationships between the physical and behavioral indices may be investigated. Two mental retardation syndromes of this type which have been simulated in animals are cretinism (e.g.Eayrs, 1961a) and phenylketonuria (e.g.Schalock & Klopfer, 1967a, b).

In humans, cretinism is characterized by a congenital dysfunction of the thyroid gland in which an impaired metabolism of iodine leads to a deficiency of thyroxine. The basic metabolic disorder is associated with anatomical evidence of an impairment of central nervous system maturation, e.g. Eayrs (1959) cites Lotmar's early work which reported the brains of cretins to be hypoplastic. In addition, Benda (1947) has identified edema and vascular changes which predispose the tissues to the type of damage characteristic of anorexia or anemic asphasia, ultimately leading to a severe loss of cells. Treatment of cretinism requires

early and sustained administration of thyroxine (Means, 1948).

In animals, cretinism can be simulated by surgical removal of the thyroid gland (Salmon, 1936), administration of methyl thiouracil (Eayrs & Taylor, 1951), or by administration of radioactive iodine (I^{131}) (Goldberg & Chaikoff, 1949). The work of Eayrs (1954, 1955, 1959, 1960 1961a, b) has established the efficacy of using the neonatally thyroidectomized rat to delineate the role of the thyroid gland in the development of nervous tissue. This work has shown that thyroid deficiency in the rat results in a retarded appearance of innately organized patterns of behavior and indicates that later in life the cretinoid rat has an impaired capacity to react adaptively to environmental change. Intellectual deficit was observed to be an inverse function of the age at which thyroidectomy was instituted (Eayrs, 1961a). These results were associated with a reduced myelination, a hypoplasia of the cell processes forming the cortical neuropile, and an altered pattern of vascularity in the rat cortex (Eayrs, 1959).

In humans, phenylketonuria (PKU) is a congenital metabolic disorder distinguished by an insufficient conversion of an essential amino acid, phenylalanine, to tyrosine due to the inactivity of the liver enzyme, phenylalanine hydroxylase (Jervis, 1953). As a result of the primary defect other abnormalities arise. Serum phenylalanine levels are raised; serum tyrosine levels are altered, and serum serotonin (5-HT) levels are lowered. The disease is accompanied generally by mental subnormality, and a progressive mental deterioration has been suggested (Nyhan, 1963). The mechanisms underlying the mental subnormality are not understood.

Knox (1960) described the central nervous system development of 26

phenylketonurics who had come to autopsy. His analysis indicated a striking lack of myelinization in the central nervous system of young patients, while this was not true for any patient over twenty-one years of age at death. Crome and Pare (1960) have also reported a myelination disorder in phenylketonurics, but they remark that the most consistent change found is a reduction in brain weight. Nyhan (1963) reports that approximately 80 percent of the phenylketonurics for whom electroencephalograms are available show some abnormalities and that cortical atrophy may be observed with pneumoencephalography. PKU is treated by restricting the dietary intake of phenylalanine. Knox (1960) has evaluated this treatment concluding that the phenylalanine-low diet is effective in preventing mental deficiency and concommitant neurologic abnormalities if it is started during the first few months of life. However, if it is initiated after three years of age, it is without effect on subsequent mental development. A recent analysis of the effects of dietary control on intelligence scores (Baumeister, 1967) supports this interpretation. The value of the treatment is questioned by some workers (cf. Birch & Tizard, 1967), however, and several problems of therapy, as well as of diagnosis, are unsolved (e.g.Hudson, 1967).

Approximately sixty studies attempting to simulate PKU in animals by administration of diets high in l tyrosine and dl phenylalanine were reviewed by Karrer and Cahilly (1965). Following a discussion of the methodological problems inherent in this mode of simulating PKU, the authors expressed their belief that claims for the experimental production of an animal analog for PKU were premature. Since that review, another method of simulating PKU has been suggested by Koe and Weissman

(1968) in a report describing the characteristics of treatment with para chlorophenylalanine (PCL). This drug strongly suppresses the tryptophane and phenylalanine hydroxylating capabilities of the rat liver while greatly raising plasma phenylalanine levels and lowering brain 5-HT levels. The authors noted that these characteristics indicate that the drug would be appropriate for production of experimental PKU.

In discussing methodological problems associated with developmental psychopharmacological investigations, Young (1967) noted that few of the learning paradigms currently used can be shown to be sensitive to otogenetic differences as evidenced by mature animals performing better than immature animals within a given species. This, he believes, handicaps investigators seeking to establish the effects of biochemical intervention on intellectual factors.

The literature is replete with studies in which acquisition measures do not vary as a function of age (eg. Stone, 1929; Verzar-McDougall, 1957; Kirby, 1963; and Doty, 1966). Most of these developmental studies used instrumental paradigms with the youngest Ss tested generally being 25 to 30 days of age. There is a great deal of evidence indicating that maturation of the rat central nervous system as measured by cortical growth parameters (eg. Sugita, 1918) as well as by biochemical and neurophysiological parameters (eg. Himwich, 1962) is completed, or virtually completed, by 25 to 30 days of age. Thus, it may be that in the majority of studies designed to relate increased age to improved learning all comparisons were made between groups of cortically mature animals which could not necessarily be expected to show differential learning capabilities.

In discussing the problems of establishing animal analogs of mental retardation, Kilbey (1969) noted that many behavioral tasks are inappropriate as measures of conditioned performance in radiothyroidectomized Ss because these animals are smaller, weaker and consume less food than normal Ss. Similar problems in experimental studies of PKU have been outlined by Karrer and Cahilly (1965). Kilbey (1969) suggested that habituation and classical conditioning paradigms which are less sensitive to performance variables related to motivational and somatic factors might offer a better assessment of intellectual deficits in experimental models of mental retardation than paradigms measuring instrumental or operant conditioned behaviors. In addition, she noted that unlike instrumental and operant paradigms, habituation and classical conditioning paradigms may be adapted easily for use with very young animals. Suggestions similar to the former have been offered by Biel and Wickens (1940) in an investigation which showed vitamin B_1 deprived rats to be inferior to normals on a conditioned eyelid reflex measure.

The developmental course of the classical conditioned response in infra-human species has been investigated only infrequently. There are, however, two reports which indicate that levels of classical conditioning may increase with increasing age. Cornwell and Fuller (1961) reported classical conditioning of the leg flexion response in puppies. They employed an air-puff conditioned stimulus (CS) and a shock unconditioned stimulus (UCS). They began conditioning trials with four day old pups, giving ten trials per day. The Ss averaged 50 percent anticipatory conditioned responses (ACRs) by 15 days of age, 70 percent by 16, and 90 percent by 19 days. In these data, age is confounded with the number of

training trials, and while the data suggest that conditioning improves gradually with age, they are inconclusive.

Gray, Yates, and McNeal (1967) reported an investigation of the classical conditioned leg flexion response in 1, 3, 6, and 12 day Ss. They measured both ACRs and test conditioned responses (TCRs). While the overall level of conditioning observed was low, maximal level ACR was 27 percent, there was a steady increase in this measure as a function of increasing age.

In summary, the information discussed has illustrated the usefulness of the experimentally analogous cretin rat in delineating the contributions of the thyroid gland to intellectual functioning. It also has indicated the lack of a satisfactory experimentally analogous phenylketonuric rat. In addition, it has pointed out the need for an experimental paradigm sensitive to differential levels of intellectual functioning as a consequence of maturational factors if the mental retardation syndromes are to be understood in terms of a general immaturity of the nervous system.

Statement of the Problem

This study was formulated to assess the usefulness of habituation and classical conditioning indices of learning for detecting any facilitating effect on learning of increased maturity, as measured by increasing age, size, and brain amine levels, in normal rats. It also attempted to determine if the learning measures differentiated normal and experimentally analogous cretin rats, a group for whom there is collateral evidence of a general central nervous system immaturity associated with learning deficits. In addition, the study undertook to establish an

experimental analog of phenylketonuria by the administration of dl para chlorophenylalanine and to assess the learning capabilities of these Ss using the habituation and classical conditioning measures.

CHAPTER II

REVIEW OF THE LITERATURE

Three research areas are pertinent to the investigation being reported: (1) studies dealing with experimental analogs of cretinism and PKU in rodents, (2) experiments in which PCL has been administered to rodents, and (3) investigations of the classical conditioned flexion response in rats. Reports of original research and review articles will be reviewed in each of these areas.

Experimental Analogs

Cretinism. A simplified method, using I^{131}, to destroy the thyroid gland of four to twelve hour old rats was reported by Goldberg and Chaikoff (1949). This method replaced the earlier surgical method (Salmon, 1936) as it had the advantages of leaving the parathyroid gland intact and lowering the experimental subject mortality rate. The I^{131} method and another, using daily administration of methyl thiouracil, have been applied to neonatal rats by Eayrs and his associates to investigate the role of the thyroid gland in mental functioning. In this way, the discrepancies arising from the use of rats made hypothyroidic as adults have been resolved (cf. Eayrs & Lishman, 1955; Eayrs & Levine, 1963).

Experimental findings based on anthropometric, neuroanatomical, neurophysiological and behavioral (innately organized and learned responses) indices were reported by Eayrs (1954, 1955 and 1959), Eayrs and Taylor (1951), Eayrs and Horn (1955), Eayrs and Lishman (1955), and Bradley,

Eayrs, and Schmalbach (1960). These studies and other clinical and experimental investigations were reviewed by Eayrs (1959, 1960) who noted that thyroid influences on the nervous system are reflected in cerebral morphology, the electrical activity of the brain, and innately organized and adaptive behavior. Indices of cerebral morphology showed a significant reduction in the length and number of dendrites developed by individual neurons, a hypoplasia of the axon network, and changes in the pattern of cortical vascularity in the neonatally hypothyrodic animal. Neurophysiological indices indicated that animals, made athyrotic at birth, manifested brain electrical activity which was reduced in amplitude with an absence of "blocking" to auditory stimulation and of "photic" driving at 36 days of age when the responses are well established in normal Ss. Using behavioral indices, neonatal thyroidectomy was shown to retard but not prevent the emergence of maturationally organized behaviors, while the capacity for adaptive behavior was severly impaired. Supporting evidence was obtained by Hamburgh,Lynn, and Weiss (1964) who reported that nonavailability of thyroxine did not influence fetal development, while it did lead to a postnatal delay in cerebrum and cerebellar maturation. Retardation of thermoregulatory mechanisms were noted as well as a performance deficit on an escape response in a water maze.

In recent investigations, Eayrs has continued to investigate the relation between age of onset of hypothyroidism and performance of a learned task. Eayrs (1961a) showed that efficiency of performance in a Hebb-Williams maze was a positive function of the age at which the rat was made athyrotic. When treatment was delayed until day 25, it did not affect performance. In addition, he showed that iodine replacement

therapy was completely successful in preventing behavioral deficits if begun by day ten but unsuccessful if delayed until day 24. Eayrs and Levine (1963) investigated the effects of neonatal and adult thyroidectomization on acquisition of a conditioned shuttle-box avoidance response. Rats treated as infants made significantly more errors than those treated as adults which, in turn, made more than untreated rats. Using this measure, replacement therapy erased the differences between the radiothyroidectomized and normal groups even though the treatment did not rectify the anatomical changes of the cerebral cortex of neonatally thyroidectomized rats. Eayrs interpreted this data as indicating that metabolic factors are the primary determinants of performance in the simple conditioned avoidance task.

Contemporaneously with this work, Eayrs and his associates have compared electrical brain activity in thyroidectomized and normal rats. Bradley, Eayrs, Glass and Heath (1961) showed that the recruiting response was characterized by an increased latency and duration and reduced amplitude in neonatally radiothyroidectomized rats, while the amplitude was not altered in adult thyroidectomized rats. The changes in temporal patterning evidenced by neonatally and adult treated Ss were reversed by replacement therapy, while the amplitude change found in neonatally treated Ss was not. These electrical activity characteristics were shown to be independent of stimulation parameters (Bradley, Eayrs & Richards, 1964). The temporal changes were postulated to arise from metabolic factors, while the amplitude change was thought to reflect a developmental growth impairment.

Eayrs (1964) hypothesized that the EEG amplitude and behavioral

changes observed in cretin animals reflect the diminished probability of axo-dendritic interaction which has been shown histologically. Campbell and Eayrs (1965) further hypothesized that work of Klee and Sokoloff (1964) and Gelber, Campbell, Deibler and Sokoloff (1964) showing that, in the course of cerebral maturation, the mitochondria of the brain lose their capacity to interact with thyroxine during protein biosynthesis may provide an underlying basis for the behavioral impairment in cretinism. The authors note that these data establish a specific relationship between thyroid hormone and protein anabolism in the nervous system. They speculate that later in life the neonatally thyroidectomized S may be unable to develop the appropriate protein determined synaptic afinities called for by some neurochemical theories of learning (cf. Hyden, 1961) and, thus, manifests an irreversible learning deficit.

Phenylketonuria. Studies, published prior to 1966, designed to investigate behavior in experimentally analogous PKU animals have been reviewed by Karrer and Cahilly (1965). The majority of these studies used rodent subjects, and in all but three PKU was simulated by use of a high phenylalanine diet, either singly or in combination with other amino acids. Two investigations used experimental groups composed of a strain of mice shown to have diminished phenylalanine hydroxylase activity and one study utilized a competitive phenylalanine hydroxylase inhibitor, dl 4-flourophenylalanine.

More recently, seven studies designed to measure intellectual and innately organized behavior in animals analogously phenylketonuric have appeared. Five used the high phenylalanine diet, one injected phenylalanine subcutaneously and one utilized an enzyme inhibitor, PCL.

Thompson and Kano (1965) report that rats were fed 10 and 6 percent dl phenylalanine and 1 tyrosine diets for a period beginning prior to pregnancy and ending at paturition. Offspring of these dams were tested at 30 to 60 days of age on an activity measure and a Hebb-Williams maze. Urine assays also were made. The urine and Hebb-Williams maze measures did not differentiate the groups, while offspring of treated mothers showed lower open field activity and higher defecation scores than those of normal mothers.

Perry, Ling, Hansen and MacDougall (1965) report two experiments in which phenylalanine (4 g/kg) singly or in combination with an equal amount of 5-hydroxytryptophan (5-HTP), a precursor of 5-HT, were given daily to newborns for 72 hours or 2.4 g/kg dosages were given for seven days. With both dosages, serum phenylalanine levels were increased and brain 5-HT levels decreased sufficiently to satisfy the biochemical requirements of simulated PKU. In addition, large amounts of tyrosine in the urine were reported. However, none of the Ss showed a learning impairment when tested on an operant discrimination task at six to eight weeks of age.

Polidor, Cunningham and Waisman (1966) examined age as a factor in the production of experimental PKU. They fed 1 phenylalanine (5 percent) diets to rats for 30 consecutive days beginning at weaning, day 20. Six other groups received this treatment for any one, or any combination, of the first three ten day postweaning intervals. A pair-fed littermate control procedure was utilized for each of the seven groups. The Ss were trained in a water maze over a six day period beginning at day 50 and on a mirror image of the maze for two days beginning at day 75.

Performance differences were not significant among the pair-fed control groups. However, each experimental group was significantly inferior to its appropriate control group when tested at day 50. In addition, groups which had received 20 or more days of treatment were significantly inferior to those which had received ten days of treatment. The deficit in maze performance was eliminated, however, when the groups were tested at 75 days of age. The authors attributed the normal performances at 75 days to the fact that the groups had been maintained on a normal diet for 20 or more days. However, one of their experimental groups which had been fed the control diet for 20 days before beginning testing at day 50 was significantly inferior to its control group. It would appear more correct to attribute the increased proficiency of the treated Ss at 75 days to an interaction of length-of-time-off-the diet and age at testing.

Schalock and Klopfer (1967b) fed groups of rats 1 phenylalanine from birth to day 60 or from day 30 to day 60. Reasoning, discrimination and discrimination reversal measures were obtained following a 23 to 65 day recovery period. Animals in the earlier and longer treated groups performed significantly worse on the discrimination task, and both experimental groups were inferior to normals on the reasoning task. There were no differences among the groups on the discrimination reversal tasks. Schalock and Klopfer (1967a) utilized a maze to measure social, locomotor, fear and exploratory behavior in the Ss reported previously. Testing, begun at day 80, revealed differences between groups on the activity measure with Ss which had been treated from birth through day 60 being significantly hypoactive.

Rendina, Ryan, De Long, Tuttle and Giles (1967) measured avoidance

learning in weaned rats fed 5 or 7 percent l phenylalanine diets and in three pups whose dam was fed a 7 percent l phenylalanine diet and who were then maintained on the diet for 18 post-weaning days. Serum phenylalanine levels were significantly increased in the experimental groups, as were the serum tyrosine levels. These Ss did not differ from their controls on the learning task.

Schlesinger, Schreiber and Pryor (1968) investigated the effects of chronic injections of PCL on a conditioned avoidance task. Injections began 24 hours after birth for rats and 48 hours after birth for mice continuing every third day until testing (41 days for rats; 21 days for mice). The PCL treated rats did not differ significantly from controls on the number of conditioned avoidance responses, but when these were considered in conjunction with escape responses PCL Ss were significantly inferior to the controls. Treated mice required significantly more trials than controls to reach an avoidance as well as an extinction criterion.

In assessing the studies published prior to 1966, Karrer and Cahilly note,

> . . . PKU as a meaningful pathology entails a relatively specific biochemical syndrome accompanied by a rather gross behavioral syndrome labeled mental retardation. One without the other cannot be PKU in any meaningful sense. Therefore, experimental attempts to produce PKU must use both adequate biochemical criteria and behavioral criteria if they are to speak of their results as PKU. There has been no study to date meeting this requirement.
>
> (Karrer & Cahilly, 1965, p. 59)

They outlined the following criteria:

Biochemical

(1) Phenylpyruvic acid in the urine

(2) Serum phenylalanine level above about 20mg percent

(3) Reduced phenylalanine hydroxylase activity

(4) Low serum tyrosine level

(5) Reduced brain and/or serum 5-HT

(6) Decreased excretion of 5-hydroxyindoleacetic acid

Behavioral

The major point the authors made was to note that since there is little information available as to the type of tasks which might discriminate the phenylketonuric individual it is necessary to measure potential intellectual deficits and behavioral abnormalities in animals with a variety of traditional measures. They further note that behavioral tasks must be chosen with regard to certain methodological problems which they outlined.

Briefly, these methodological problems involve adequate control, motivational, developmental and species-difference factors. In terms of adequate controls, the major problem has been that ingestion of substances which alter phenylalanine metabolism has given rise to extremely high mortality rates. Animals treated with these substances voluntarily restrict their dietary intake which has often resulted in significantly smaller experimental animals than their littermate controls. In terms of motivational variables, the reduced food intake of the experimental Ss invalidates the use of hours of deprivation to insure motivation and food to insure reward. Likewise, the grossly different sizes make comparison of speed measures inappropriate, and the use of shock to establish avoidance behavior may be contaminated by skin resistance changes

produced by unbalanced diets. Insofar as developmental variables are concerned, they note that studies utilizing Ss which are first treated in adolescence or adulthood are inappropriate since the clinical evidence points to irreversible damage to the central nervous system due to an abnormal chemical mileau during the early developmental stages. In discussing species differences they review evidence that phenylalanine hydroxylase activity in primates is comparable to that of humans while that of the rat is not and, therefore, suggest that work with primates is best suited to this problem.

Assessment of the more recent studies in terms of the criteria suggested by Karrer and Cahilly shows that they too failed to establish both a biochemical and a behavioral simulation of PKU. Performance decrements, indicative of intellectual deficit, were not found by Thompson and Kano (1965), Perry, et al (1965), or Rendina, et al (1968). Biochemical measures were not obtained by Polidor, et al (1966) or by Schlesinger, et al (1968), and PKU was not indicated by the urine measure obtained by Thompson and Kano (1965). The Rendina, et al (1968) study reported significantly increased tyrosine levels which are uncharacteristic for the older phenylketonuric. As most of the studies using this method have reported increased urine or serum tyrosine levels (eg. McKean, Schanberg & Giarman, 1962; Perry, et al, 1965) and none have reported lowered tyrosine levels, the validity of the Schlock and Klopfer (1967a, b) studies must also be questioned. Presumably all studies using high phenylalanine diets violate the physiological model of PKU since this treatment increases serum tyrosine levels, while in humans these levels are shown to be significantly decreased after 11 months of

age (Partington & Lewis, 1963).

Para Chlorophenylalanine Studies

Biochemical. Koe and Weissman (1966) administered various derivatives of PCL to male, adult rats at four drug levels (10, 32, 100 and 316 mg/kg) for three successive days previous to obtaining biochemical measures. They report that PCL considerably depleted brain 5-HT and brain 5-hydroxy-3-indoleacetic acid, decreased brain norepinephrine (NE) levels slightly and inhibited liver tryptophane hydroxylase and phenylalanine hydroxylase. Plasma PCL plus phenylalanine levels were increased and plasma tyrosine levels decreased. Dl and l derivative of PCL were the more effective in inhibiting liver tryptophane hydroxylase in vitro while all derivatives were equally effective in reducing brain 5-HT. The authors suggested that PCL depleted brain 5-HT by inhibiting its biosynthesis by blocking tryptophane hydroxylation.

Lipton, Gordon, Guroff and Udenfriend (1967) reported that adult rats given an initial dosage of PCL (300 mg/kg) followed 72 hours later by an additional 100 mg/kg showed increased phenylalanine brain and plasma levels and decreased tyrosine levels. Liver phenylalanine hydroxylase was markedly inhibited. The authors noted that only in animals treated with PCL does the tyrosine content of the blood remain low even though the phenylalanine concentration is markedly elevated.

Jequier, Lovenberg and Sjoerdsma (1967) gave single injections of PCL (300 mg/kg) to rats and varied the time of sacrifice. They reported a high correlation between 5-HT depletion and the degree of tryptophane hydroxylase inhibition which supported Koe and Weissman's (1966) hypothesis that 5-HT depletion results from enzyme inhibition. The exact

mechanism of tryptophane hydroxylase inhibition by PCL is undetermined, however.

Behavioral. Koe and Weissman (1966) reported that treatment of rats with PCL (three successive daily doses of 100 mg/kg) which lead to virtually total depletion of 5-HT elicited no obvious behavioral effects in that there was no evidence of sedation, hyperactivity, ptosis, temperature change or rotorod performance disruption. The total overt behavioral response consisted only of a possible mild irritability. Weissman noted that 90 to 95 percent reductions in brain 5-HT did not disrupt performance of a previously established avoidance response (cited in Tenen, 1967).

Tenen (1967) utilized male, adult rats administered PCL (100 mg/kg) for three days or this dosage plus 5-HTP (75 mg/kg, administered one hour before testing). Acquisition of conditioned avoidance (two levels of shock), conditioned emotional, or position habit responses were measured. Pain sensitivity, emotionality (open field test), and activity measures also were obtained. The PCL group showed a faster acquisition of an active avoidance response at the low current level of shock. Since this group had a lower jump threshold for shock than controls, increased pain sensitivity would seem to account for their faster acquisition. With shock levels above jump threshold for all groups, no differences in performance were noted. The PCL group showed less disruption on the conditioned emotional task, while the groups did not differ in acquisition of a position habit. Neither emotionality measures nor activity measures differentiated the groups.

Stevens, Resnick and Krus (1967) used three discrimination tasks to

test male, adult rats given PCL (316 mg/kg) on the second pretraining day and every third day, thereafter. The PCL group learned simultaneous and successive discrimination tasks with fewer errors than controls. No differences were found between the groups in the position discrimination and discrimination reversal tasks.

Rosen and Buga (1968) administered 5-HTP, cinanserin (a 5-HT antagonist), and PCL (three successive daily doses of 100 mg/kg) to four male rats. Impaired fixed ratio reinforced lever pressing response was reported following PCL administration.

Stevens, Fechter and Resnick (1968) reported the effects of a single injection of PCL (316 mg/kg) on a passive avoidance response and open field behavior tested three days post injection. The PCL treated Ss required significantly more trials to reach an avoidance criterion. In the open field test, significantly fewer PCL treated Ss urinated and they were significantly less active. The groups did not differ in the number of contacts with novel stimuli.

In addition to the Schlesinger data previously discussed, the authors (Schlesinger et al, 1968) also investigated the effects of a single PCL injection (320 mg/kg) on a conditioned pole-climbing avoidance task using three levels of shock and two strains of rats. On the conditioned avoidance response the PCL treated Ss were superior to the controls, while total responses (avoidance plus escape) did not differentiate the groups. Treated Ss of one strain were superior to their controls at low and medium levels of shock, while the other strain was superior at the high level.

Welch and Welch (1967) showed that mice pretreated with PCL (360mg/kg)

failed to show normal increases in brain 5-HT when exposed to the stress of fighting and restraint. Fighting behavior normally observed when previously isolated mice are placed together was also absent in the treated group.

Kilbey, Harris and Aalund (1969) treated rats from birth through day 23 with dl PCL and obtained 65.5 percent depletion of 5-HT and a plasma phenylalanine level of 17.8 mg percent. Eleven weeks post drug administration, 5-HT levels and body weights, which had been significantly lowered during treatment, were normal. These Ss were tested for activity and for the extinction-induced rate increase phenomena, which is considered a measure of aggression (cf. Thompson, 1961, 1962). There were no differences in activity measures between the groups, while the extinction ratio for the dl PCL groups was significantly lower than that of the control group, indicating a reduction in aggression. The authors also reported that at maturity total brain weights of the dl PCL early treated Ss were significantly lower than those of control Ss.

Some of the above studies support Koe and Weissman's (1968) suggestion that PCL treatment would be an appropriate method for establishing a PKU analog while others do not. In general, the production of biochemical indices of PKU with PCL treatment has been a consistent finding (Lipton, et al 1967; Jequier, et al, 1967; Kilbey, et al, 1969). In addition, the Kilbey, et al (1969) report of lower brain weight for early treated PCL Ss established that this treatment produces the most common neuroanatomical pathology of the human phenylketonuric (Crome & Pare, 1960). Moreover, the reports of irritability and hypersensitivity to pain have their counterparts in the clinical disease (Koe & Weissman,

1968; Tenen, 1967). As PKU patients often show hyperactivity, fearfulness and an uncontrollable temper (Knox, 1960), conflicting evidence arises from those studies reporting that PCL treatment decreases emotionality (Tenen, 1967; Stevens, et al, 1968), aggression (Welch & Welch, 1967; Kilbey, et al, 1969) and activity (Stevens, et al, 1968).

The learning data cannot be considered relevant to the establishment of a PKU analog since they are based on acute administration of PCL to adult animals.

The facilitating effect of PCL on active avoidance responding has been considered to result from hypersensitivity to pain in PCL treated Ss due to 5-HT depletion (Tenen, 1967). That hypersensitivity to pain is due to 5-HT depletion and not other effects of PCL administration is supported by the report that the phenomena can be produced when 5-HT is lowered by a completely different method (Harvey & Lints, 1965). The Schlesinger, et al (1968) report that the shock level which facilitated performance varied from low to high current levels with different strains would seem to complicate this interpretation. However, intrastrain differences in endogenous 5-HT levels as well as differences in response to monoamine oxidase inhibitors have been reported (Miller, Cox & Maickel, 1968). In the absence of investigations of intrastrain responses to PCL, the relationship between 5-HT depletion, pain sensitivity, and the facilitory effect of PCL on active avoidance tasks cannot be determined.

The deleterious effect of PCL treatment on passive avoidance performance has been interpreted by postulating that PCL causes a decreased emotionality. In turn, this facilitates some tasks, eg. brightness

discrimination learning, by protecting the S from frustration factors postulated to operate in this task (Stevens, et al, 1968). However, to relate these phenomena to 5-HT mechanisms (cf. Stevens, et al, 1968) in the absence of collateral evidence is not warranted given the numerous effects of PCL on biochemical systems.

Classical Conditioning of the Flexion Response

There are very few investigations of classical conditioned flexion responses in the rat. Schlosberg (1934) reported individual records of classical conditioned tail movements obtained from rats. The report focused on the problems encountered in obtaining the measure. The author concluded that a satisfactory method had been developed in the preliminary work reported. The study did not demonstrate classical conditioning nor report its absence, however, as the controls necessary to establish this fact were not undertaken.

A similar report by Schlosberg (1936) covers four procedural investigations of classical conditioning of the leg flexion response in the rat. As both the previous investigations had shown too low an incidence of leg or tail conditioned responses to warrant an experiment, Kappauf and Schlosberg (1937) undertook another procedural investigation of the conditioned leg flexion response in which they varied the duration of the CS, shock-response contingencies and other factors. Conditioned leg flexion levels were reported to be less than eight percent. The authors concluded,

> Considering the series of papers as a whole, it may be seen that many experimental factors have been varied but no set of conditions has yet been found under which a specific withdrawal of the rat's foreleg may be consistently elicited by a new stimulus (light or buzz) associated during training

> with the shock. It now seems clear that the general situation is unfavorable for such conditioning with the white rat.
> (Kappauf & Schlosberg, 1937, p. 44)

In weighing this conclusion, one should consider the fact that in the most standardized investigation conducted by these authors, six durations of CS and two shock-response contingencies were varied, and twelve rats were used, e.g. one for each condition. The degree to which these data account for the observation that no investigation of these responses seems to have appeared in the literature during the following 25 year period is an interesting conjecture.

Chacto and Lubow (1967) reported successful conditioning of the tail flexion response in 85 day old rats. A tone served as the CS (1 sec.) and shock (175 msec., 1.5 mA) as the UCS. The inter-trial interval (ITI) was randomized (40, 60 and 80 sec.) and four days of acquisition with one day of extinction were completed (50 trials per day). Acquisition ACRs for the CS-UCS paired, CS-UCS unpaired, and CS alone groups were subjected to analysis of variance which yielded significant treatment and treatment by day effects. The maximal ACR level (approximately 70 percent) for the CS-UCS paired groups was found on day two.

Although no recent studies of classical conditioning of the leg flexion response in adult rats are available, it has been established that neonatal Ss are capable of acquiring this response. Caldwell and Werboff (1962) tested rats during the first eight hours after birth using a vibro-tactile CS (128 cps) and a shock UCS (1.0 mA, 50 msec.). Four CS durations were used (300, 600, 1200 and 2400 msec.) and eight blocks of ten trials were completed. A pseudoconditioning group for each CS duration was used. These groups did not differ from one another on the ACR

measure. The ACRs for the 600, 1200 and 2400 msec. groups were significant when compared with the combined control groups and also when compared with the 300 msec. group. In discussing their results, the authors noted that the maximal level of neonatal conditioning observed (32 percent) was considerably lower than that traditionally reported for adult Ss on conditioning tasks. In addition, they noted that the highest mean level of conditioning was manifested by the 1200 msec. group, while the best interstimulus interval (ISI) for learning in adult Ss is between 300 and 600 msec. They hypothesized that the longer optimal ISI for their Ss is associated with the low degree of myelinization and low speed of neural conduction of the neonatal period.

Caldwell, Brand, and Werboff (1962) report an experiment using similar parameters except that the CS durations were 900 and 1800 msec. Two levels of environment temperature (28 degrees and 23 degrees C) were maintained. Pseudoconditioning control groups were maintained for each experimental group. The authors report that performance differences between experimental and control groups indicated the validity of the conditioning data. Within the experimental groups, 28 degrees C Ss were superior to 23 degrees C Ss. The highest performance level (29.8 percent) was attained by the 1800 msec., 28 degrees C group. These data indicate that conditioning levels in poikilothermic rats are significantly altered by environmental temperature.

Gray, et al (1967) have extended this work with an investigation of the classical conditioned leg flexion response in one, three, six and twelve day old rats. In this experiment ambient temperature was maintained at 90 degrees F, and three CS durations (300, 600 and 1200 msec.)

were used. Each training block consisted of nine pairings of CS-UCS and one test trial of CS alone. Both ACRs and TCRs were measured. At six and 12 days, significantly higher ACRs were obtained in the 600 and 1200 msec. groups than in their control groups. The TCR measures showed significantly higher levels for 600 and 1200 msec. groups at three days of age and for the 300 msec. group at 12 days. These authors also interpreted their data as indicating that longer CS durations facilitate conditioning in young animals.

These studies have shown that longer CS durations facilitate performance in neonatal and young rats. However, to relate this finding to the immaturity of the neonatal central nervous system seems unwarranted for several reasons. First, there are no data on this task for the adult rat; second, the most comparable study of adult rats (Chacto & Lubow, 1967) used an invariant 1000msec. CS, and third, while shorter CS durations (e.g. 500$\pm$ 250 msec.) are reported as optimal for adult, human Ss, the optimal duration for conditioning in adult, animal Ss would seem to be two seconds (Hall, 1964). In any event, the question of the interaction between CS duration and conditioning level as a function of age beyond 12 days must be resolved empirically.

CHAPTER III

METHOD

Design

The study was designed to investigate habituation and classical conditioning of the leg flexion response in rats during the cortical developmental period as a function of maturation and differential drug treatment. Previous investigations (Caldwell & Werboff, 1962; Gray, et al, 1967) have shown that conditioning levels for the leg flexion response in rats under 13 days of age are low in comparison with those generally obtained from adult Ss on conditioning tasks. It is hypothesized that the low level of conditioning observed in young Ss is related to the relative immaturity of the rat cortex and that these levels could be expected to increase during the period of cortical maturation (birth to 30 days) in the rat. To test this hypothesis, normal rats (T4 groups) were tested at four age levels, A1 (7 days), A2 (14 days), A3 (21 days), and A4 (28 days), during this period. A pseudoconditioning control group (T5) was included at each age level. The T4 and T5 Ss were sacrificed after conditioning and serum phenylalanine and brain 5-HT and NE measures were obtained.

Koe and Weissman (1968) have suggested that PCL is an appropriate drug for establishing an animal analog of PKU. To test this hypothesis, two groups (T1 and T2) were administered different dosages of dl PCL from birth until 72 hours prior to testing at the four age levels. Serum phenylalanine and brain 5-HT and NE were measured to establish the effects

of dl PCL on Ss of this age range and to establish the presence of biochemical indices of PKU in these groups.

The work of Eayrs and associates (e.g. Eayrs, 1960) has provided neuroanatomical, neurophysiological and anthropometric evidence of pathology in rats neonatally thyroidectomized associated with an impaired performance on learning tasks. However, no information is available on the learning performance of these Ss at young ages or on a classical conditioning task. For these reasons a group of neonatally radiothyroidectomized Ss (T3) was included at each age level. The biochemical measures were obtained for this group, and in addition, thyroid gland tissue was histologically examined to determine the effectiveness of the I^{131} treatment.

Drug control groups (T6 and T7) were maintained on dl PCL schedules identical to those of T1 and T2 groups. These groups were sacrificed 24 hours after the last drug administration in order that the acute effects of dl PCL could be compared with those obtained from T1 and T2 groups which were sacrificed after completing the habituation and conditioning tasks, e.g. 86 hours post-drug administration. A normal control group (T8) was also sacrificed at this time, and the measures were obtained.

Each group consisted of ten Ss. The age range was three days; four Ss on the day designating the age level and three on both the preceding and the following day.

Subjects

The 320 Ss used in the study were bred from 72 LEW/f Mai female and 24 LEW/f Mai male albino rats purchased from Microbiological Associates, Walkersville, Maryland. The LEW/f Mai is an inbred strain, F_{47}, and the

animals purchased represented 12 sets of six sisters and four brothers. The rats were housed in double cages with three sisters and one brother to a cage. When delivery was imminent, females were transferred to individual cages. When three or more females gave birth within an eight hour period, the litters were split, and individual litters were randomly assigned to the various treatment conditions. From birth until 20 days of age, the individual litters were kept in the breeding cages. From that point on, they were housed in colony cages. Mothers and young had free access to Wayne Mouse Breeder Blox, phenylalanine content 1.01 percent, and water at all times. No attempt was made to wean experimental Ss and all Ss were observed to be unweaned at 29 days. However, Ss were observed to eat food and drink water available in the home cage after, approximately, the 20th day of age.

Procedure

Treatment. Differential group treatments are displayed in Table 1. The dl PCL was suspended in .20 percent agar and intubated. For Ss under 25 grams, polyethylene tubing, size 10, and 27 gauge needles were used. For Ss above 25 grams, intubations were given with size 20 tubing and 25 gauge needles. The length of tubing used for neonates was 3 cm., and this was increased in 1/2 cm. steps as the Ss grew. Intubated volume was 10 ml/kg. In order to maintain body temperature, intubation procedures for nonambulating Ss were carried out with the S positioned on a warm heating pad.

Preparation. To obtain the pre-conditioning, habituation and conditioning measures, the Ss were positioned in one of the following ways. The Ss under 25 grams weight were taped to a board of sufficient width

TABLE 1

DAILY TREATMENT SCHEDULE

Group	Birth to Day 13	Day 13 to Day 27
T1	150 mg/kg dl PCL	100 mg/kg dl PCL
T2	100 mg/kg dl PCL	100 mg/kg dl PCL
T3*	.20 percent agar	.20 percent agar
T4	.20 percent agar	.20 percent agar
T5	.20 percent agar	.20 percent agar
T6	150 mg/kg dl PCL	100 mg/kg dl PCL
T7	100 mg/kg dl PCL	100 mg/kg dl PCL
T8	.20 percent agar	.20 percent agar

* 150 microcuries of I^{131} injected subcutaneously within eight hours of birth

to support the stomach and head, tapered to allow free movement of the legs. The end of the board supporting the S's head was clamped to a ring stand. The Ss over 25 grams were placed on a two or three inch rubber covered ring on a ring stand using rubber bands which went under the legs and over the back.

A reference electrode of soft lead of sufficient size to encircle the torso and cover three-fourths of the shoulder to pelvis area, was moistened with E & M electrode paste. It was placed around the S and secured by tape. A six mm. circular lead shock electrode was moistened with E & M electrode paste and attached by tape to the upper portion of the S's left leg.

A 12 inch nylon surgical thread was tied to the right leg and attached to an E & M myograph. Type A myographs, zero to three grams pressure sensitivity were used for Ss weighing less than approximately 20 grams, and type B, 0 to 30 grams pressure sensitivity, were used for Ss over 20 grams. Myographs were calibrated before each S was tested so that maximum pressure caused a six cm. pen deflection on the E & M physiograph record. Vibro-tactile stimulation (120 cps) was delivered through a curved glass rod to which the S's tail was affixed by tape.

The A1 Ss underwent testing in an incubator (Chicago Surgical & Electrical Company, catalog number 300) at 32 degrees C. For older Ss the ambient temperature was maintained at approximately 27 degrees C. The Ss were tested in a small, isolated room which was not soundproof but was fairly quiet. During the pre-conditioning procedure two Es were present. During habituation and block one of the conditioning procedure, one E was present. Thereafter, the S was alone and was checked

during each interblock interval.

Pre-conditioning procedure and measures. The general experimental paradigm is shown in Appendix A. During the pre-conditioning period several measures were obtained in the order indicated: (1) skin resistance was read from the shock and reference electrodes using a Simpson voltmeter, (2) maximal size of spontaneous leg flexion (SLF) was determined (three samples), (3) the current level necessary to elicit a minimal (1 mm.) pen deflection was determined, (4) the current level necessary to elicit three successive deflections equal to the S's maximal SLF was determined, and (5) the S's response to five presentations of each of twelve intensity levels of vibration was determined. The various intensity levels were presented at 15 second intervals in the random order indicated in Appendix B.

For measures three, four and five E1 manipulated the shock or vibro-tactile intensity, while E2 judged the response. For measure five, deflections of one mm. and over were considered responses. The highest intensity of vibro-tactile stimulation to which the S responded not more than 40 percent of the time was designated as the CS intensity level. The current value obtained by measure four was designated as the UCS level.

Habituation procedure and measure. The CS was presented every 20 seconds. Any discernible pen deflection during the 1000 msec. presentation was considered a response. If there were evidence of movement prior to the CS onset, the presentation was not scored. The number of trials necessary to reach an habituation criterion of ten successive presentations with no discernible response was recorded. Seven Ss did not

habituate and were discarded. They were as follows: one T1/A1 S, three T2/A2 Ss, one T3/A2 S, one T4/A1 S, and one T4/A3 S.

Conditioning procedure and measures. The conditioning paradigm consisted of an initial three minute period followed by eight blocks of ten paired CS-UCS presentations and a test block of ten CS presentations. The procedure for determining the CS and UCS intensity has been outlined. Occasionally an S would be observed to react differently to the UCS intensity during conditioning than during the UCS determination procedure. Two types of responses were noted: (1) the S responded with very vigorous multiple leg flexions, or (2) the S responded with leg flexions considerably below his maximal leg flexion. If the first three responses to the UCS during conditioning fell in either of these classes, shock was adjusted over the next two or three trials to yield a maximal leg flexion. The CS duration was 1000 msec., and UCS duration was 50 msec. Offset of the CS was followed by onset of the UCS. The interval between successive CS-UCS pairings for conditioning groups followed the schedule included in Appendix C and averaged 60 seconds. The random schedule of CS and UCS presentations for pseudoconditioning groups is included in Appendix D. For both paradigms the inter-block interval was three minutes. Following conditioning, Ss were marked by clipping the ear and returned to the home cage.

All discernible SLFs observed in the three minute period preceding block one and block eight were scored as a measure of general activity. During the training blocks, deflections occurring after the onset of the CS and before the onset of the UCS and which were of three mm., or greater, amplitude and 300 msec., or longer, duration were scored as ACRs.

The same criteria were used during the test block to score TCRs.

Biochemical procedures and measures. Each morning, all appropriate drug control Ss and the conditioning Ss from the preceding day were sacrificed. These Ss were guillotined. Blood samples and the brain were removed and frozen. In 14 T3 and 14 T4 Ss the thyroid glands were removed and placed in formalin.

Whole brain 5-HT and NE were measured fluorimetrically. The number of brains, within a group, which constituted a sample varied with age of the Ss. Brain tissue was homogenized in two parts 0.01 N HCl and extracted from a salt saturated solution with butanol at pH 2.0. This simultaneously removed both 5-HT and NE (Wiegand & Perry, 1961). Following butanol extraction for one hour on the automatic shaker, the 5-HT and NE were returned to an aqueous phase by the addition of excess heptane. The 5-HT was read directly on an aliquot of the aqueous phase by measuring at the 540 wavelength in a strong acid solution with a modified number five arrangement (Wise, 1967a, b). The NE was determined by forming the trihydroxyindole derivative with iodine as the oxidizing agent and stablizing the product with alkaline ascorbic acid ethylenedramine reagent (von Euler & Lishajko, 1961).

Plasma phenylalanine was determined fluorimetrically. To prepare a protein-free solution 0.6 N trichloroacetic acid (Reagent F) was added to the sample. This was mixed well and centrifuged for approximately five minutes. To each blank, standard and sample tube 0.30 ml of ninhydrinpeptide (Reagent G) was added. All tubes were placed in a constant temperature water bath at 60 degrees C for two hours. The tubes were then cooled with a tap water bath for approximately three minutes. One

and one-half ml dilute copper solution (Reagent H) was added to each tube and mixed by inversion several times. The percent transmission of each tube was then read from the fluorimeter at zero (Sigma Chemical Co., 1966).

William Schindler, Ph.D., Baylor University College of Medicine, Houston, Texas, administered the I^{131} injections to neonatal T3 Ss and completed the histological examination of the thyroid tissue obtained from T3 and T4 Ss.

Apparatus

A schematic representation of the system used is provided in Appendix E. The system was controlled by a punch tape on the tape programmer (Ralph Gerbrands, Model 1A) number one on the diagram. Individual tapes were available for the conditioning, vibro-tactile determination, and habituation procedures. Individual tapes were used for CS and UCS delivery in the pseudoconditioning paradigm. As the tape moved, a pin dropped into a hole, and a switch was closed which allowed the relay designated K to energize. One set of relay contacts allowed the timer (Hunter Manufacturing Co., Model 11C) to operate. The other set of contacts provided an operate for the pulse former.

One set of timer contacts put the signal from the audio generator (The Heath Co., Model 1GW72) into the impedance matching transformer designated T1 which caused the speaker (QUAM, Model 4A07738) and the attached glass rod to vibrate for one second.

The pulse former operated the stimulator (Grass Instrument Co., Model S4CR). For conditioning, the stimulator delay was set for one second and provided at the offset of the speaker vibration an output to

the constant current unit (Grass Instrument Co., Model CCUIA). This delivered a 50 msec. shock to the S.

For pseudoconditioning, switch one on the auxiliary chassis was placed in position two. The system operation was the same except that the pulse former was now operated by the tape programmer designated two. The stimulator was operated independently and the pulse former provided a slash for the event marker on the physiograph (E & M Instrument Co., Inc., Model MKII, Ser. No. 362). In all paradigms, CS onset and offset were recorded by the physiograph event marker.

Tape programmers, timer and relay K were located in an adjoining room. The remainder of the equipment was located in the room in which the Ss were tested.

CHAPTER IV

RESULTS

Survival rates were calculated for the first 310 Ss born. These rates were: .79 (T4, T5, and T8 groups), .73 (T1 and T6 groups), .68 (T2 and T7 groups), and .63 (T3 groups). It should be noted that the change to a lower drug dosage on day 13 for T1 and T6 groups was predicated on the finding that less than five percent of these Ss survived if the 150 mg/kg treatment were carried beyond day 17.

Data for the nine measures obtained during the pre-conditioning, habituation and conditioning paradigms were subjected to a Cochran Test for Homogeneity of Variance (Winer, 1962). The hypothesis of homogeneity of variance was rejected for three measures: basal skin resistance ($C_{.99} = .1655$), current to elicit minimum leg flexion ($C_{.99} = .3350$), and number of trials to reach an habituation criterion ($C_{.99} = .2148$). These data were analyzed using the Kruskel-Wallace Non-parametric Analysis of Variance (Siegel, 1956). All other data were analyzed using a version of Balanova 5, a general analysis of variance program for the IBM 7090 developed by Paul Herzberg, York University. Individual comparisons were made using the Tukey (a) procedure (Winer, 1962). Unless noted differently, all results designated as significant refer to p less than .01.

Comparison of the number of TCRs yielded significant treatment and age effects (Table 2). Individual comparisons of mean TCRs for the treatment groups showed T4 groups to be superior to all other treatment

TABLE 2

ANALYSIS OF VARIANCE

TEST CONDITIONED RESPONSES

Source	df	MS	F Ratio
Treatment (T)	4	61.454	14.41*
Age (A)	3	22.899	5.37*
T X A	12	8.274	1.94
Subjects	180	4.262	

* p less than .01

groups (Table 3). Individual comparisons of TCRs for T4 groups as a function of age showed the A3 group to be superior (p less than .05) to the A1 and A2 groups, while they did not differ significantly from the A4 group (Table 4). Figure 1 shows the mean number of TCRs for the five conditioning groups. Figure 2 shows these data as a function of age, and Figure 3 displays the mean number of TCRs for each treatment group as a function of age. These data strongly support the hypotheses that conditioning is demonstrable, that it increases as a function of age during the cortical developmental period for normal Ss, and that the treatments would result in an impaired conditionability for experimentally analogous PKU and cretin groups.

Analysis of variance computed for the ACR data yielded significant treatment, age and block effects as well as significant treatment by age, treatment by block, age by block, and treatment by block effects (Table 5). The mean ACRs for the five treatment groups are shown in Figure 4. Figure 5 shows the mean ACRs for the four age levels. The mean number of ACRs as a function of block is shown in Figure 6. Treatment and block mean ACRs are plotted in Figure 7, while the age and block mean ACRs are shown in Figure 8. These data also support the hypotheses investigated.

Age and treatment by age effects were significant in the analysis of the number of SLFs preceding block one (Table 6). The mean number SLF for each treatment group is displayed in Figure 9. Figure 10 contains the mean number SLFs for each age level, and Figure 11 shows the age by treatment relationship. The data indicate that activity, as measured by SLFs, did not vary among the groups prior to initiating

TABLE 3

TUKEY (a) TEST OF TREATMENT MEANS:

TEST CONDITIONED RESPONSES

TREATMENTS	T1	T5	T3	T2	T4
T1 (PCL-H)					*
T5 (Pseudo)					*
T3 (I^{131})					*
T2 (PCL-L)					*
T4 (Nor Cond)					

* p less than .01

TABLE 4

TUKEY (a) TEST OF AGE MEANS:

T4 TEST CONDITIONED RESPONSES

AGE	A1	A2	A4	A3
A1 (7 day)				*
A2 (14 day)				*
A4 (28 day)				
A3 (21 day)				

* p less than .05

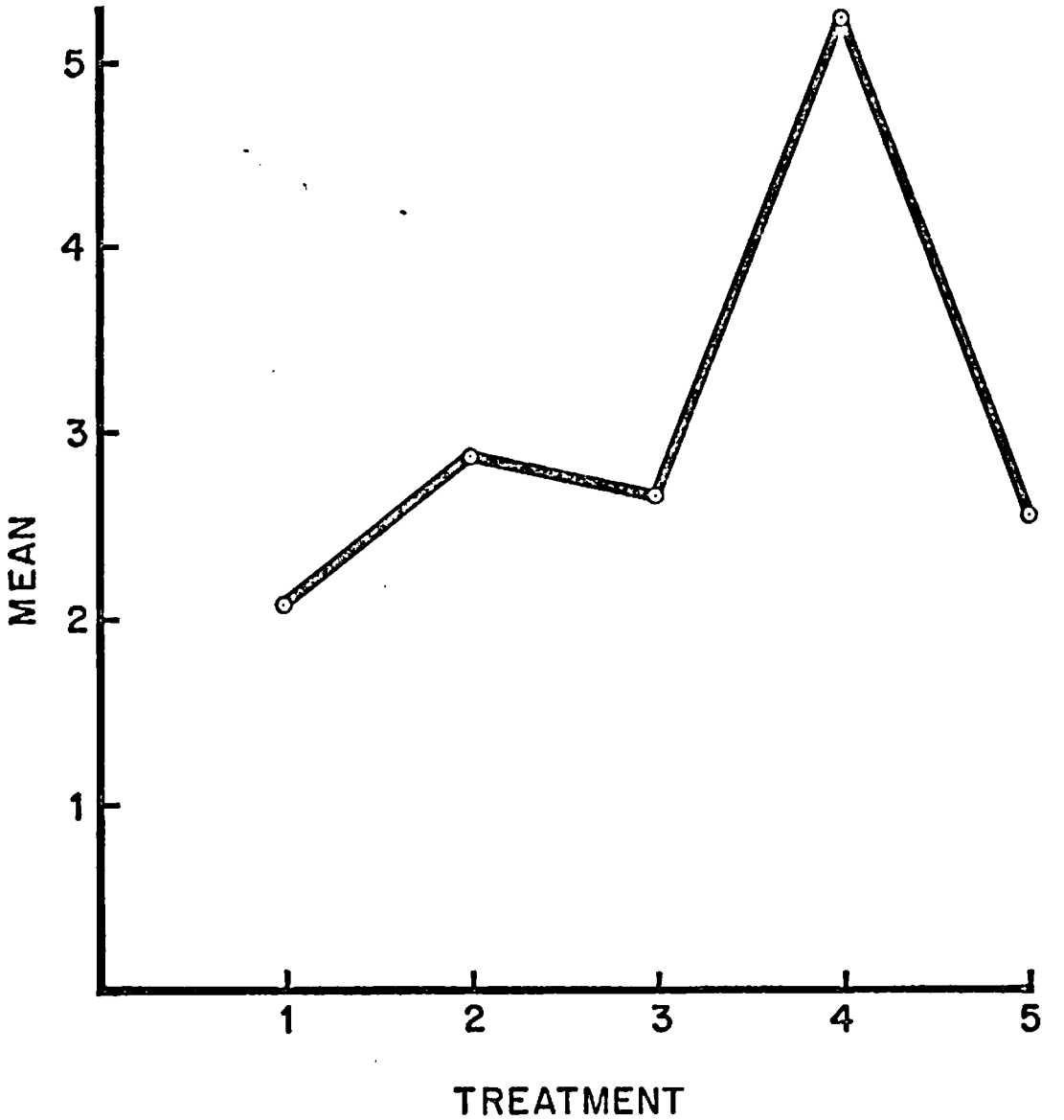


FIGURE 1

MEAN TEST CONDITIONED RESPONSES FOR FIVE TREATMENT GROUPS

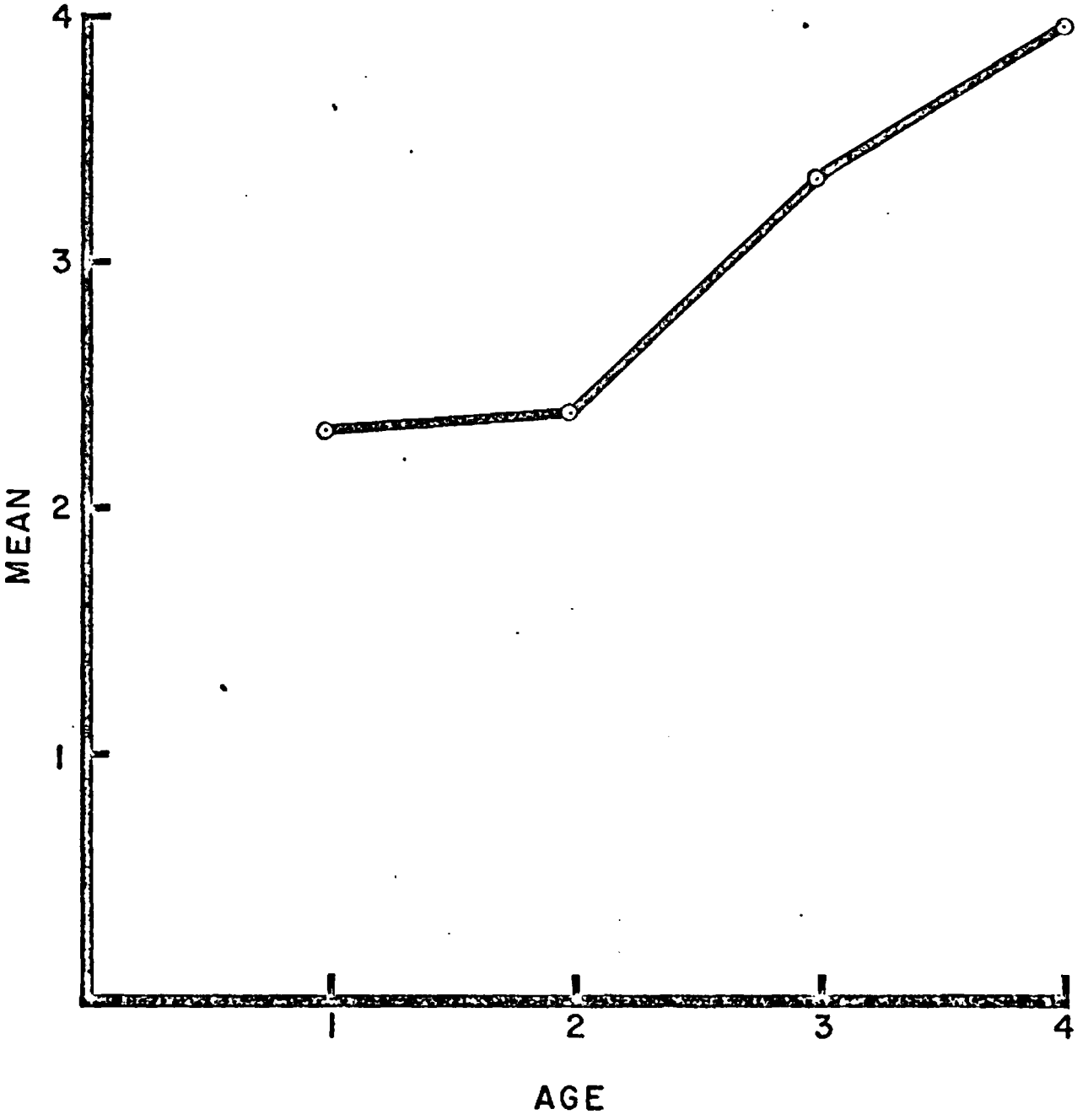


FIGURE 2

MEAN TEST CONDITIONED RESPONSES

FOR FOUR AGE LEVELS

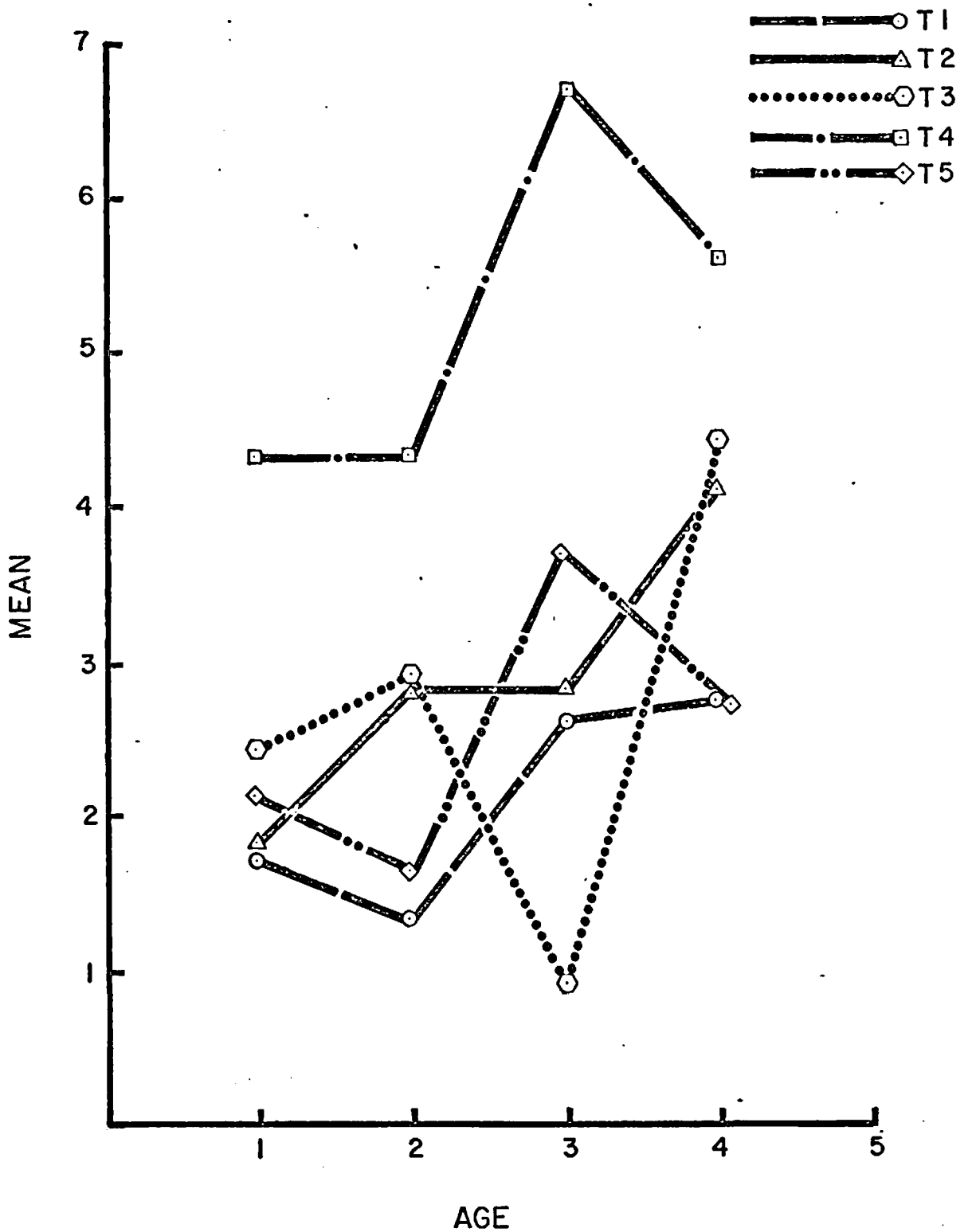


FIGURE 3

MEAN TEST CONDITIONED RESPONSES

FOR FIVE TREATMENT GROUPS AT FOUR AGE LEVELS

TABLE 5

ANALYSIS OF VARIANCE:

ANTICIPATORY CONDITIONED RESPONSES

Source	df	MS	F Ratio
Treatment (T)	4	209.767	11.44*
Age (A)	3	142.185	7.76*
T X A	12	45.106	2.46*
Subject (S)	180	18.322	
Block (B)	7	51.218	20.50*
T X B	28	6.023	2.41*
A X B	21	4.787	1.91*
T X A X B	84	3.220	1.28
S X B	1260	2.497	

* p less than .01

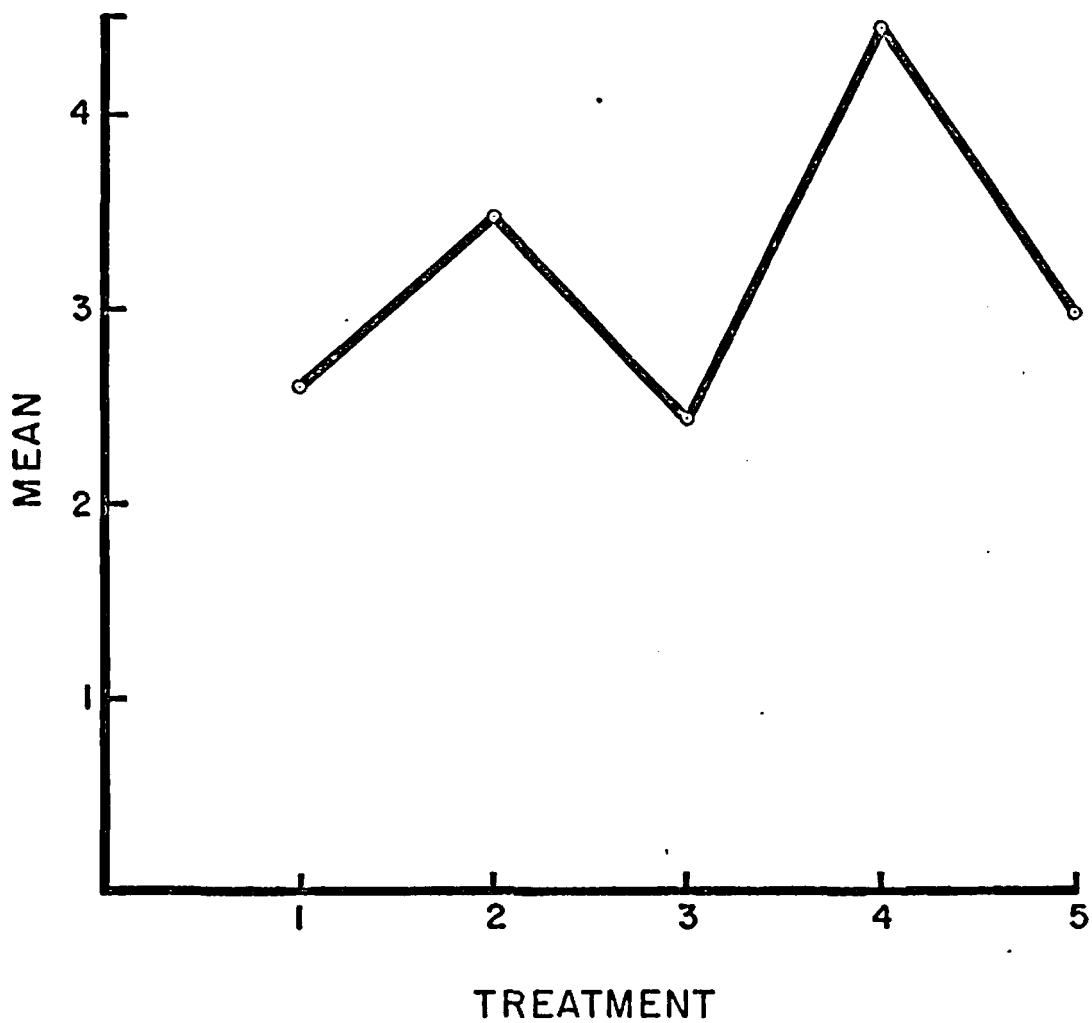


FIGURE 4

MEAN ANTICIPATORY CONDITIONED RESPONSES

FOR FIVE TREATMENT GROUPS

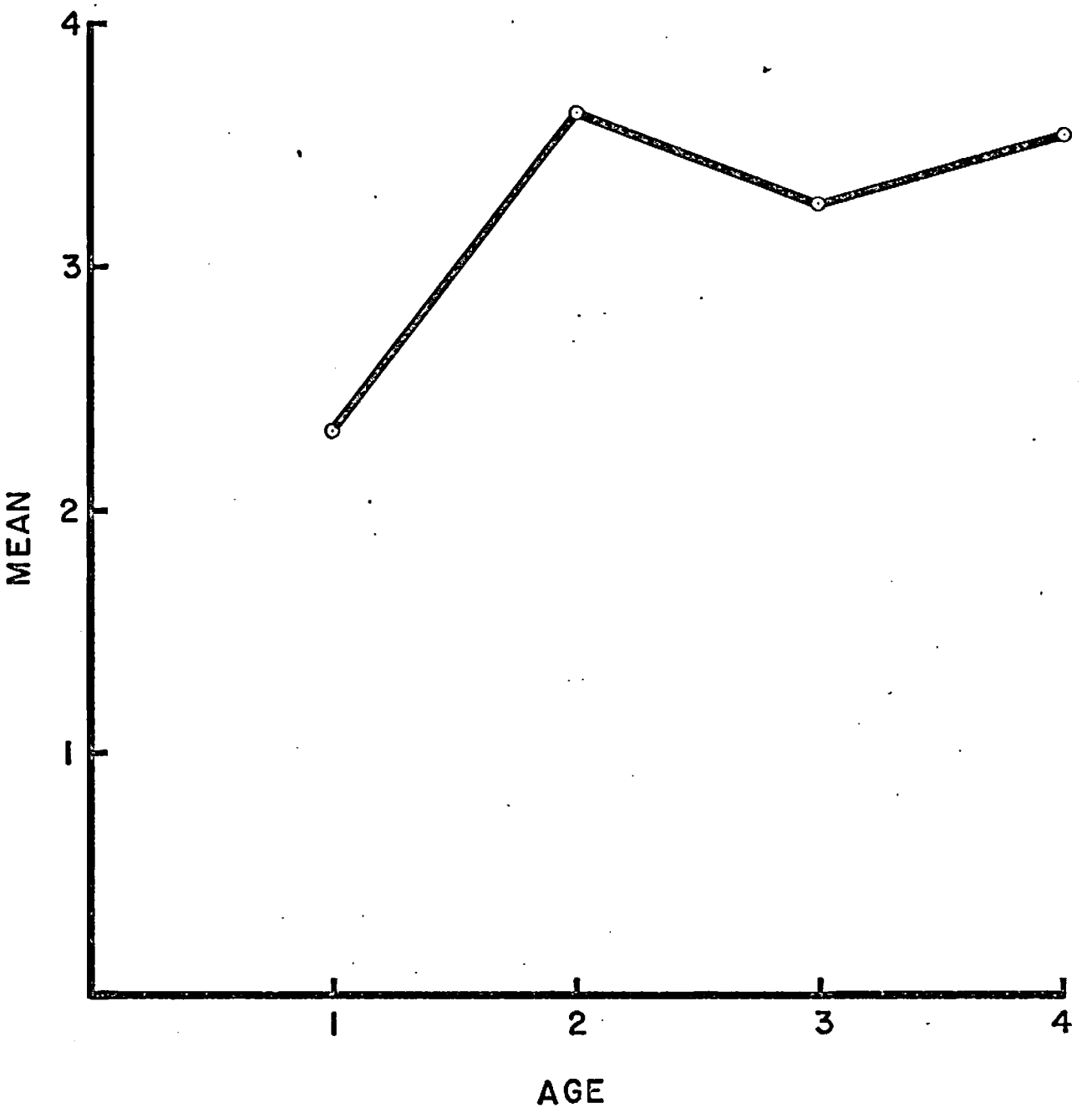


FIGURE 5

MEAN ANTICIPATORY CONDITIONED RESPONSES

FOR FOUR AGE LEVELS

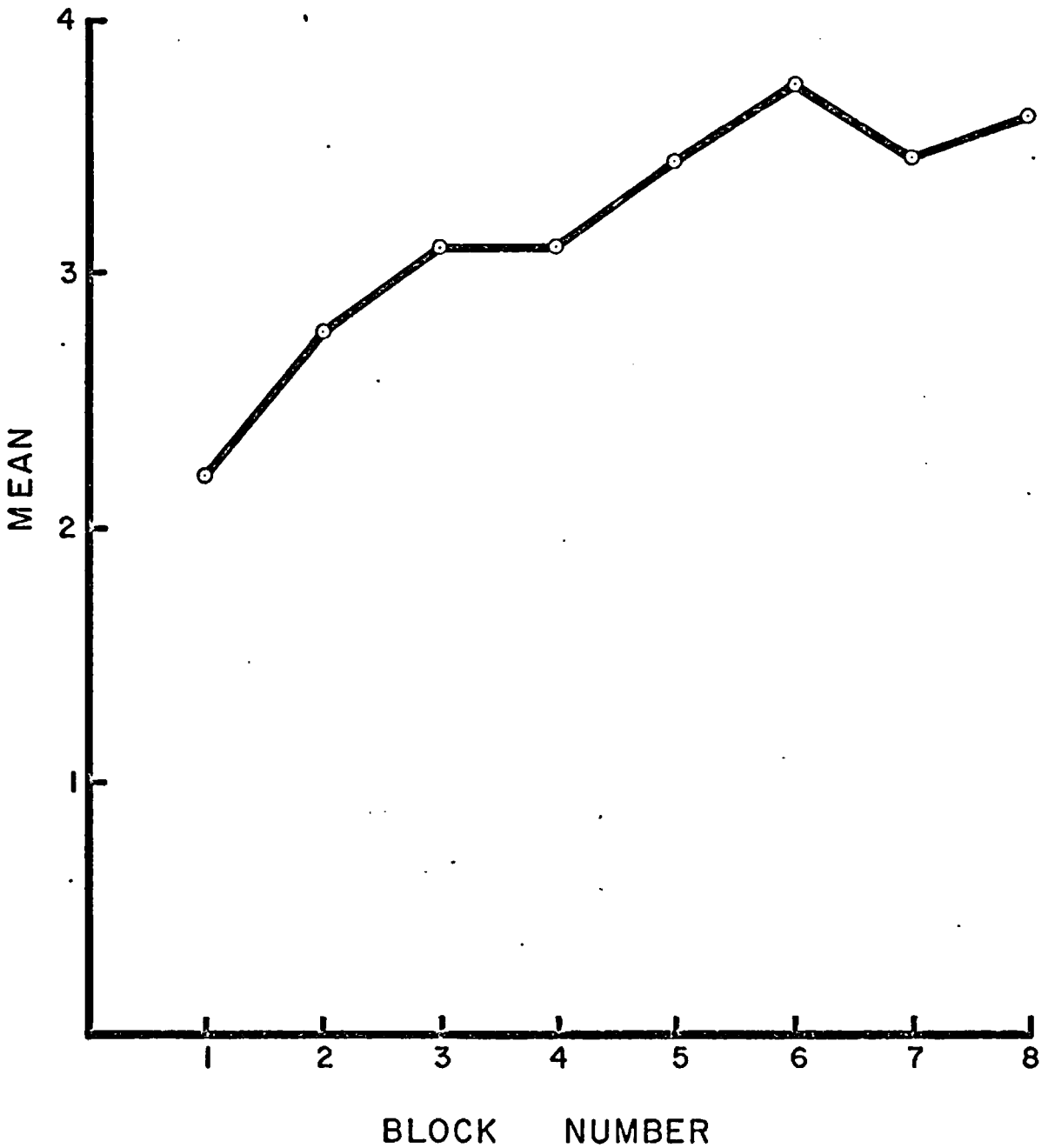


FIGURE 6

MEAN ANTICIPATORY CONDITIONED RESPONSES

FOR EIGHT BLOCKS OF TRIALS

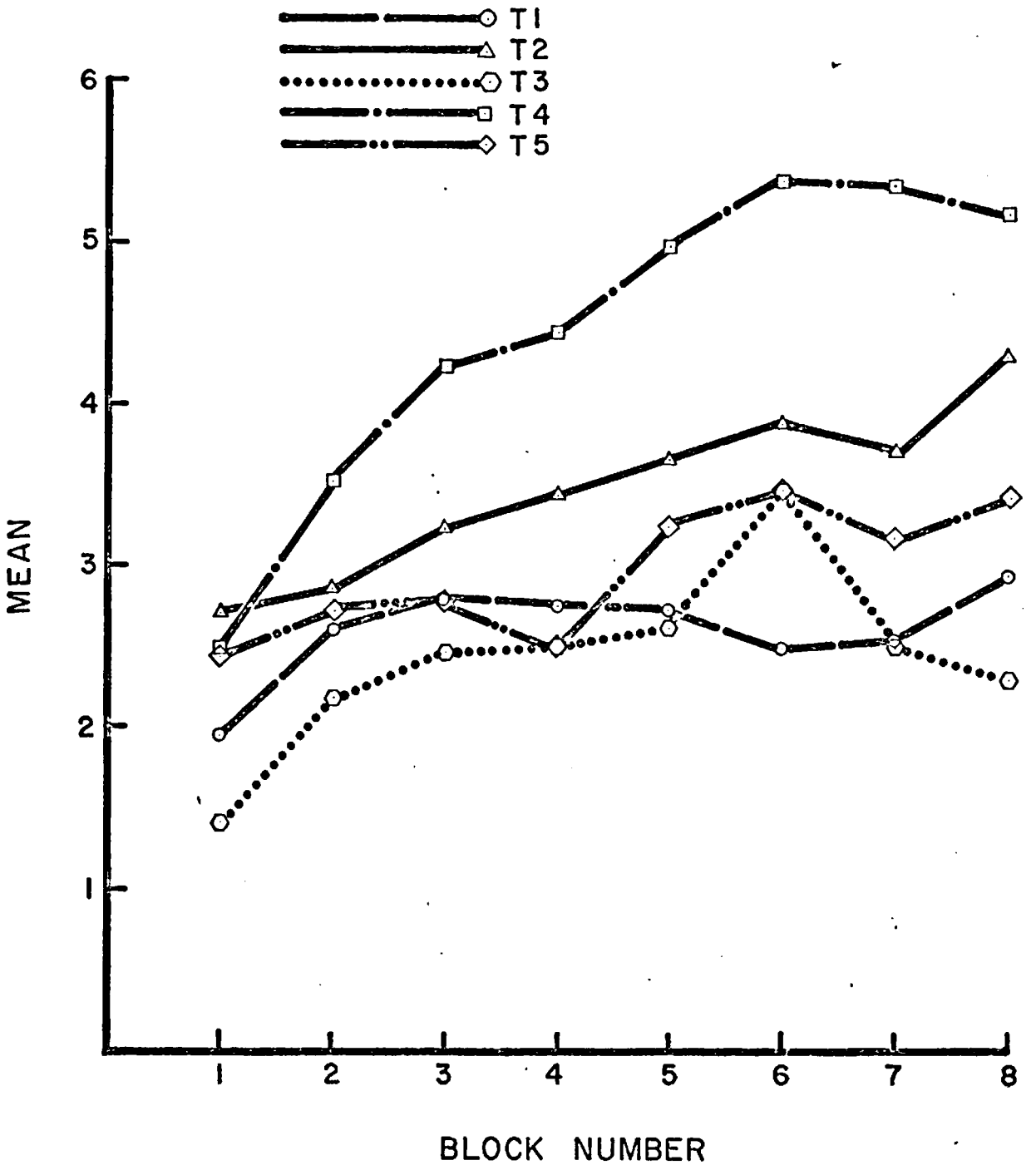


FIGURE 7

MEAN ANTICIPATORY CONDITIONED RESPONSES

FOR FIVE TREATMENT GROUPS OVER EIGHT TRIAL BLOCKS

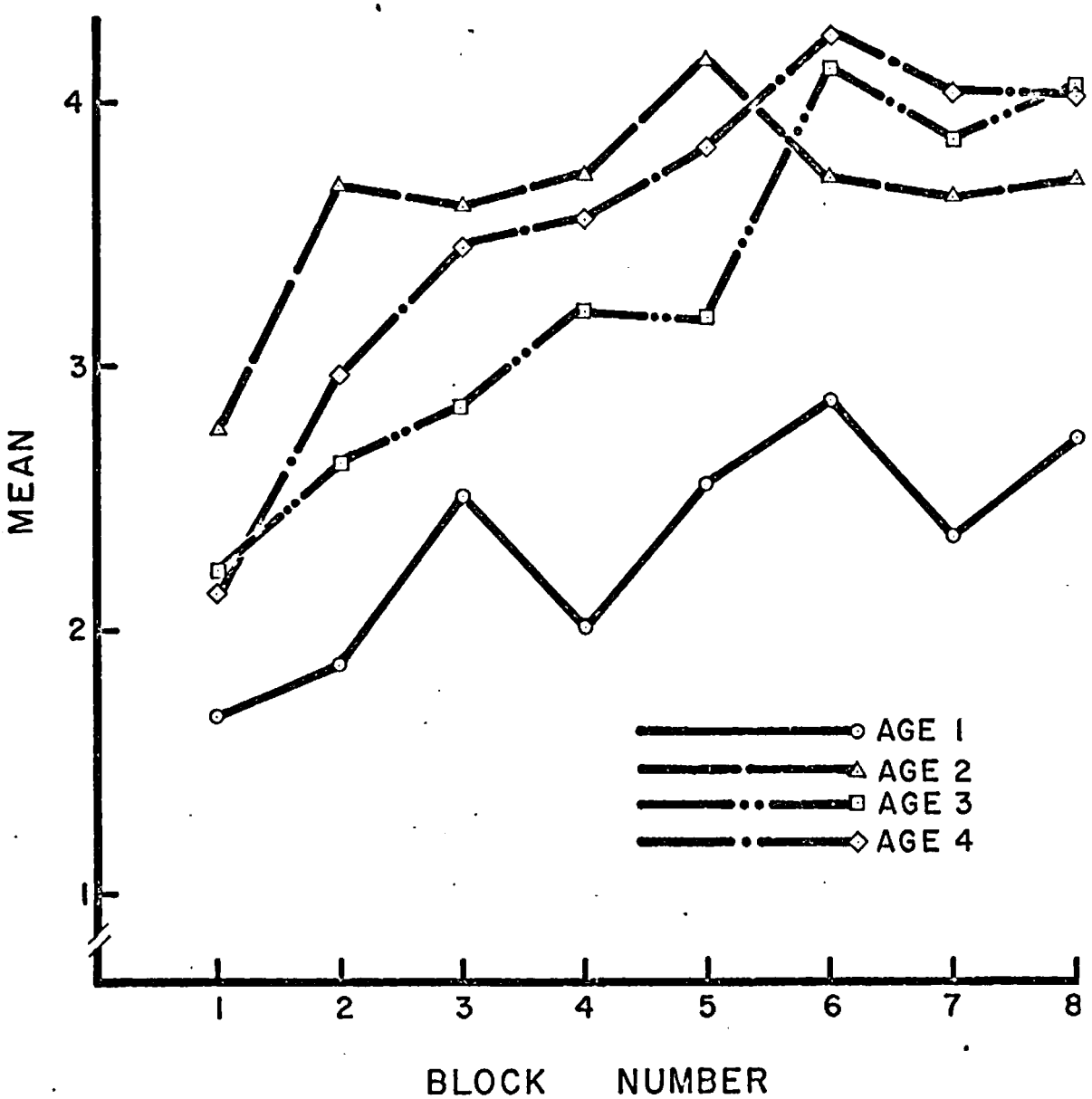


FIGURE 8

MEAN ANTICIPATORY CONDITIONED RESPONSES

FOR FOUR AGE LEVELS OVER EIGHT TRIAL BLOCKS

TABLE 6

ANALYSIS OF VARIANCE:

SPONTANEOUS LEG FLEXIONS PRECEDING BLOCK ONE

Source	df	MS	F Ratio
Treatment (T)	4	2114.732	2.28
Age (A)	3	8968.497	9.67*
T X A	12	2509.885	2.70*
Subjects	180	926.786	

*p less than .01

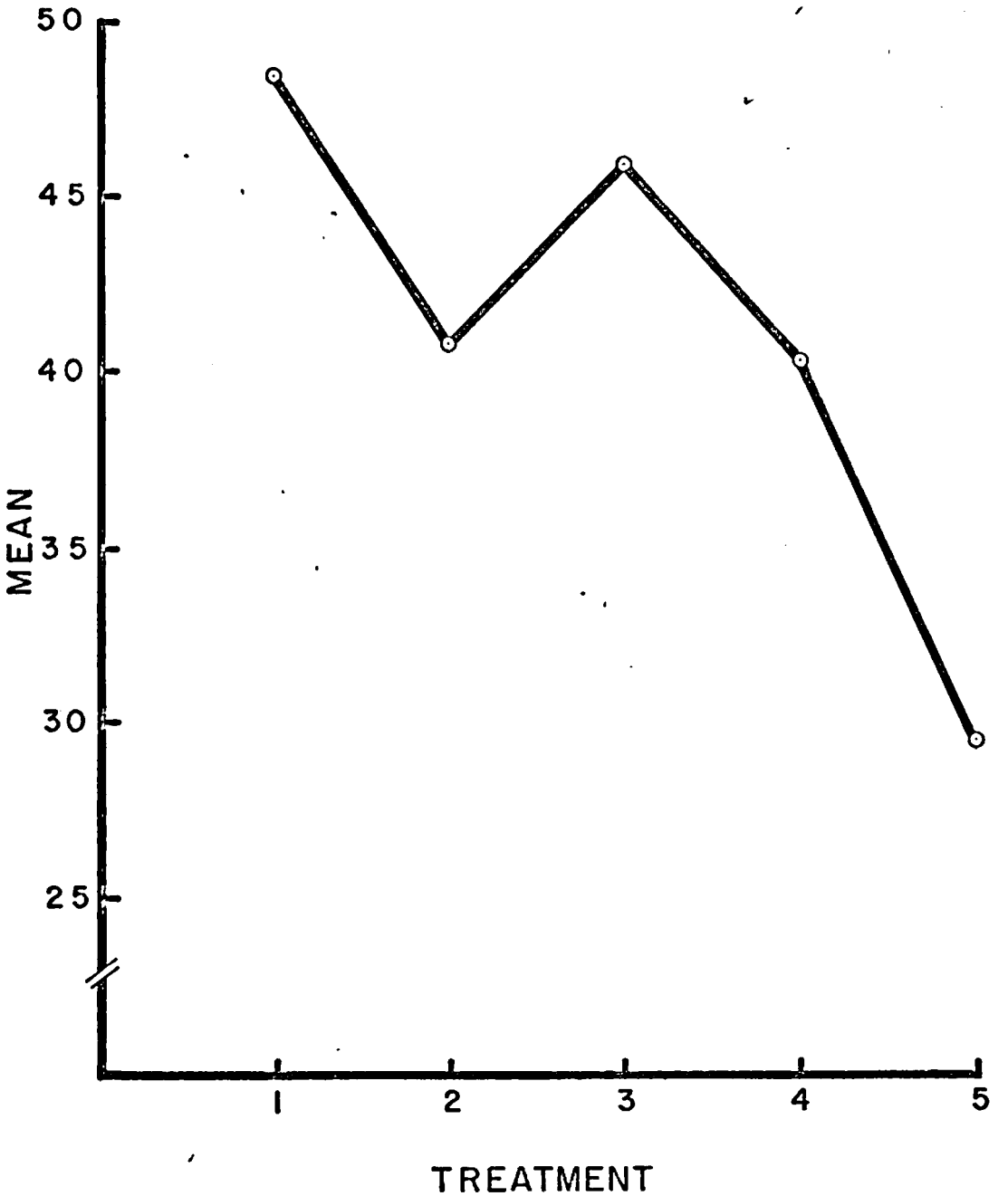


FIGURE 9

MEAN SPONTANEOUS LEG FLEXIONS PRECEDING TRIAL BLOCK ONE FOR FIVE TREATMENT GROUPS

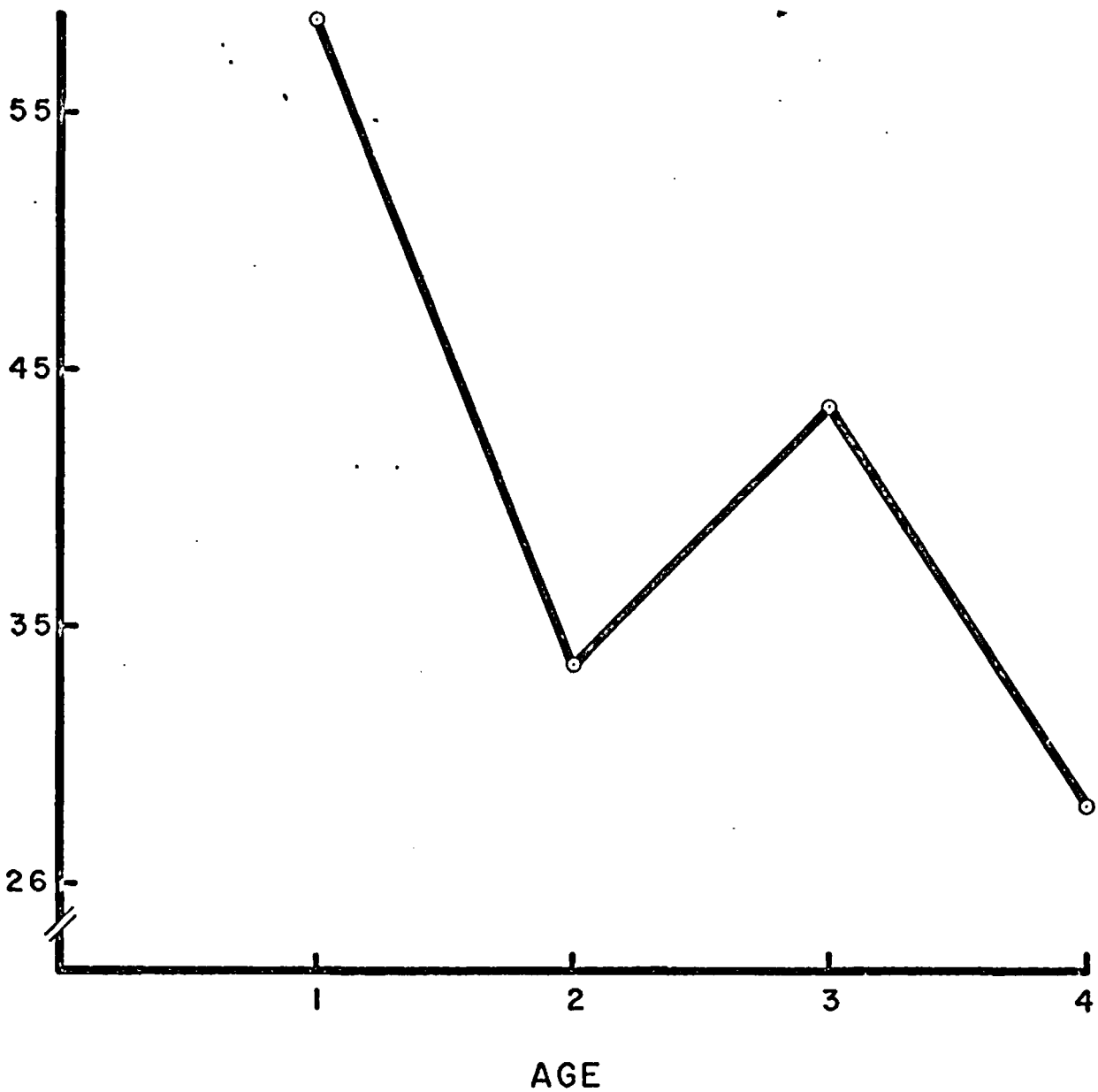


FIGURE 10

MEAN SPONTANEOUS LEG FLEXIONS PRECEDING TRIAL BLOCK ONE

FOR FOUR AGE LEVELS

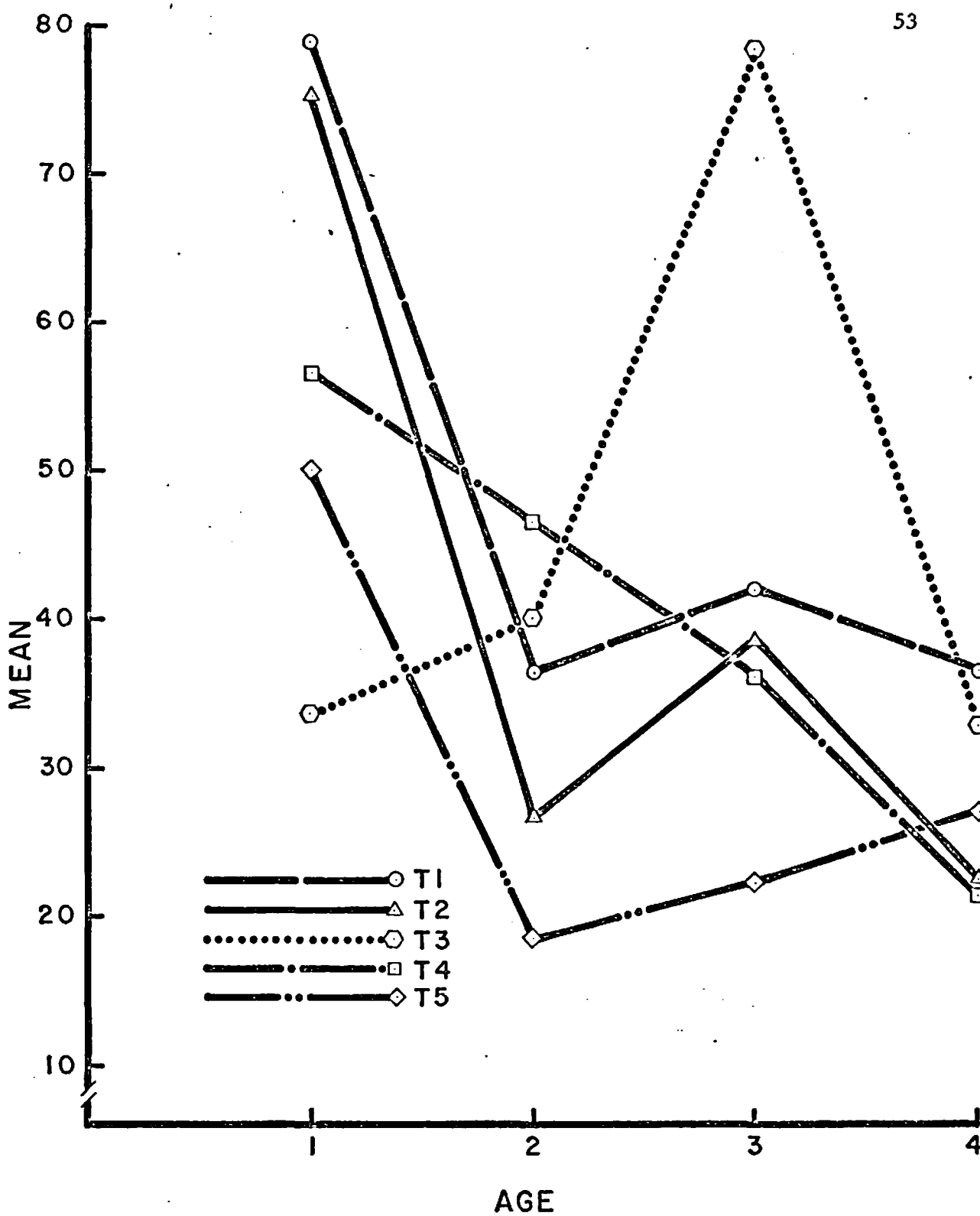


FIGURE 11

MEAN SPONTANEOUS LEG FLEXIONS PRECEDING TRIAL BLOCK ONE

FOR FIVE TREATMENT GROUPS AT FOUR AGE LEVELS

conditioning trials, and that activity declined during the developmental period. The significant age by treatment interaction reflects the rise in SLFs found for the I^{131} groups at 14 and 21 days which was not characteristic of the other groups at 14 days and which was considerably less for other groups at 21 days.

Analysis of the number of SLFs preceding block eight yielded significant treatment, age and treatment by age effects (Table 7). Figure 12 shows the mean SLFs preceding block eight for the five treatment groups, while Figure 13 gives the same information for the four age levels. These data indicate that activity preceding conditioning block eight was greatest for dl PCL (high dosage) and I^{131} groups. Activity for all groups decreased sharply at 14 days and remained relatively stable thereafter. The age by treatment interaction followed that outlined previously and is not displayed. The basal skin resistance data yielded an H of 18.60 ($p < .01$) indicating that the treatment conditions were associated with differential basal skin resistance levels. The mean basal skin resistance levels for the treatment groups are shown in Figure 14. Figure 15 shows the mean basal skin resistance for the four age levels. These data are plotted for each treatment group at each age in Figure 16. These data indicate that basal skin resistance levels of I^{131} groups are higher than other groups. Figure 15 illustrates that basal skin resistance levels fell sharply from seven to 14 days and remained stable thereafter.

Analysis of the grams pressure of SLF yielded significant treatment, age, treatment by age and treatment by age by order (e.g., first, second, or third flexion recorded) effects (Table 8). The average grams pressure

TABLE 7

ANALYSIS OF VARIANCE:

SPONTANEOUS LEG FLEXIONS PRECEDING BLOCK EIGHT

Source	df	MS	F Ratio
Treatment (T)	4	4179.517	3.79*
Age (A)	3	20660.696	18.74*
T X A	12	1410.443	1.27
Subjects	180	1102.364	

* p less than .01

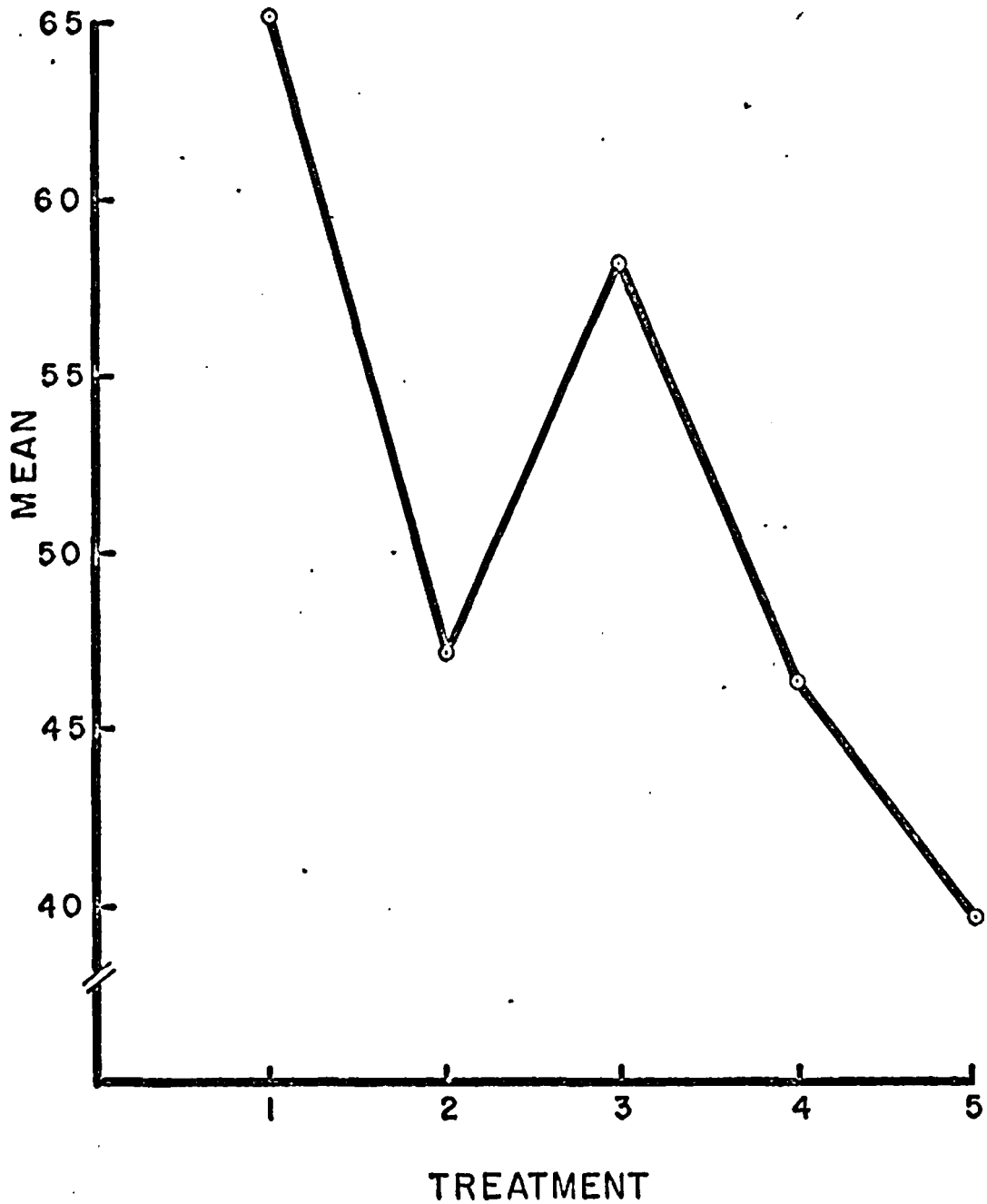


FIGURE 12

MEAN SPONTANEOUS LEG FLEXIONS PRECEDING BLOCK EIGHT

FOR FIVE TREATMENT GROUPS

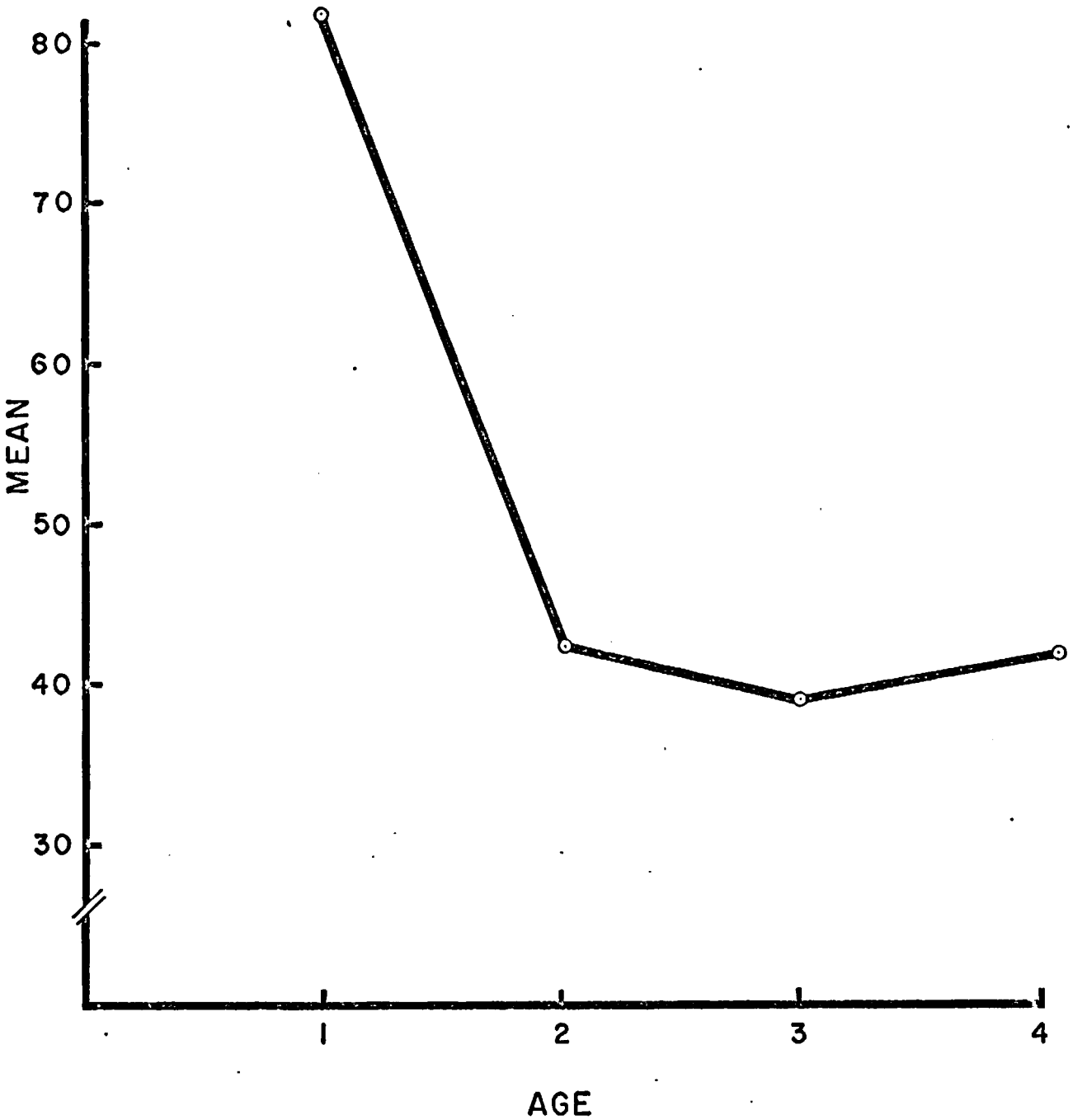


FIGURE 13

MEAN SPONTANEOUS LEG FLEXIONS PRECEDING BLOCK EIGHT

FOR FOUR AGE LEVELS

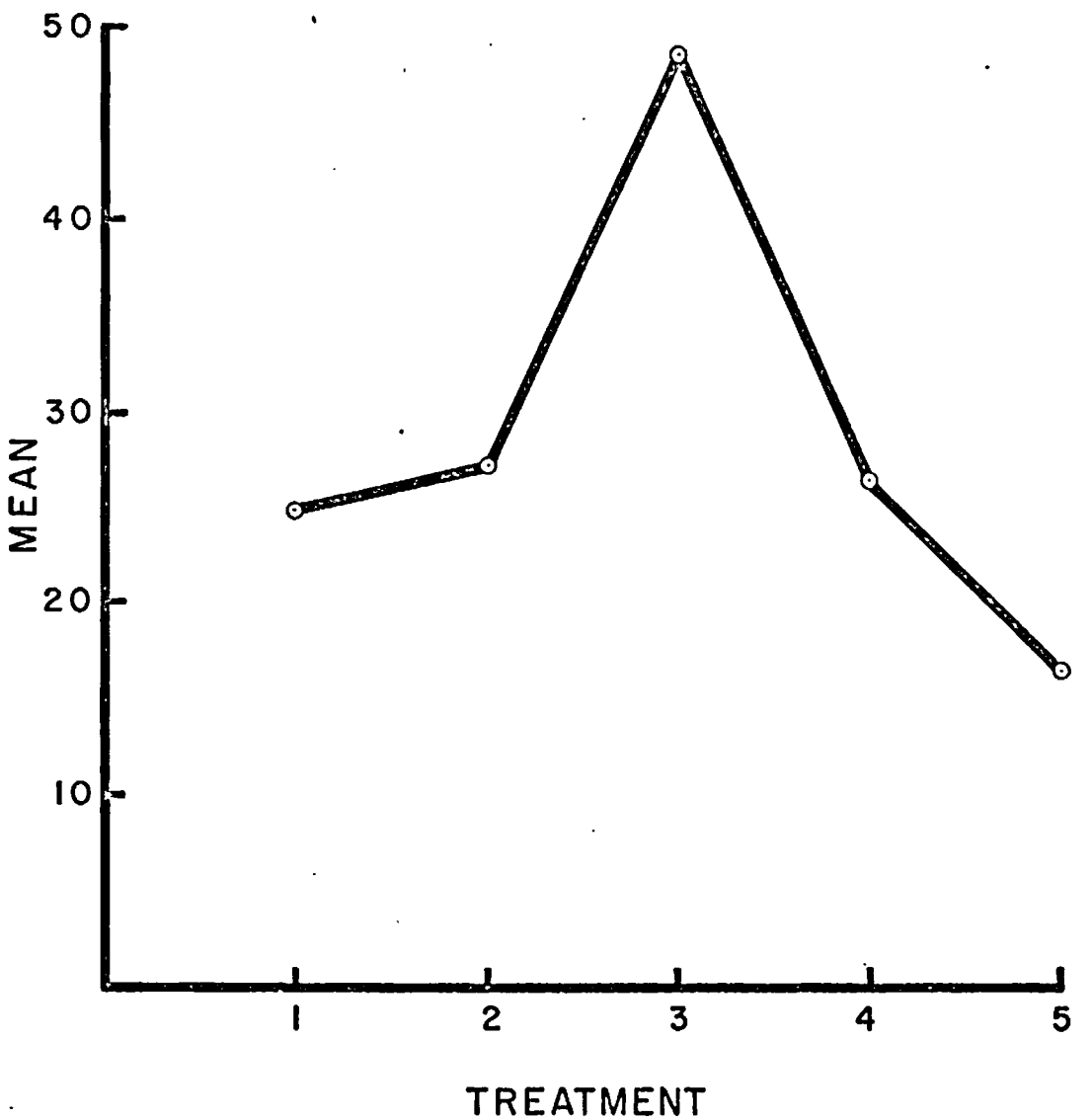


FIGURE 14

MEAN KILOHM BASAL SKIN RESISTANCE

FOR FIVE TREATMENT GROUPS

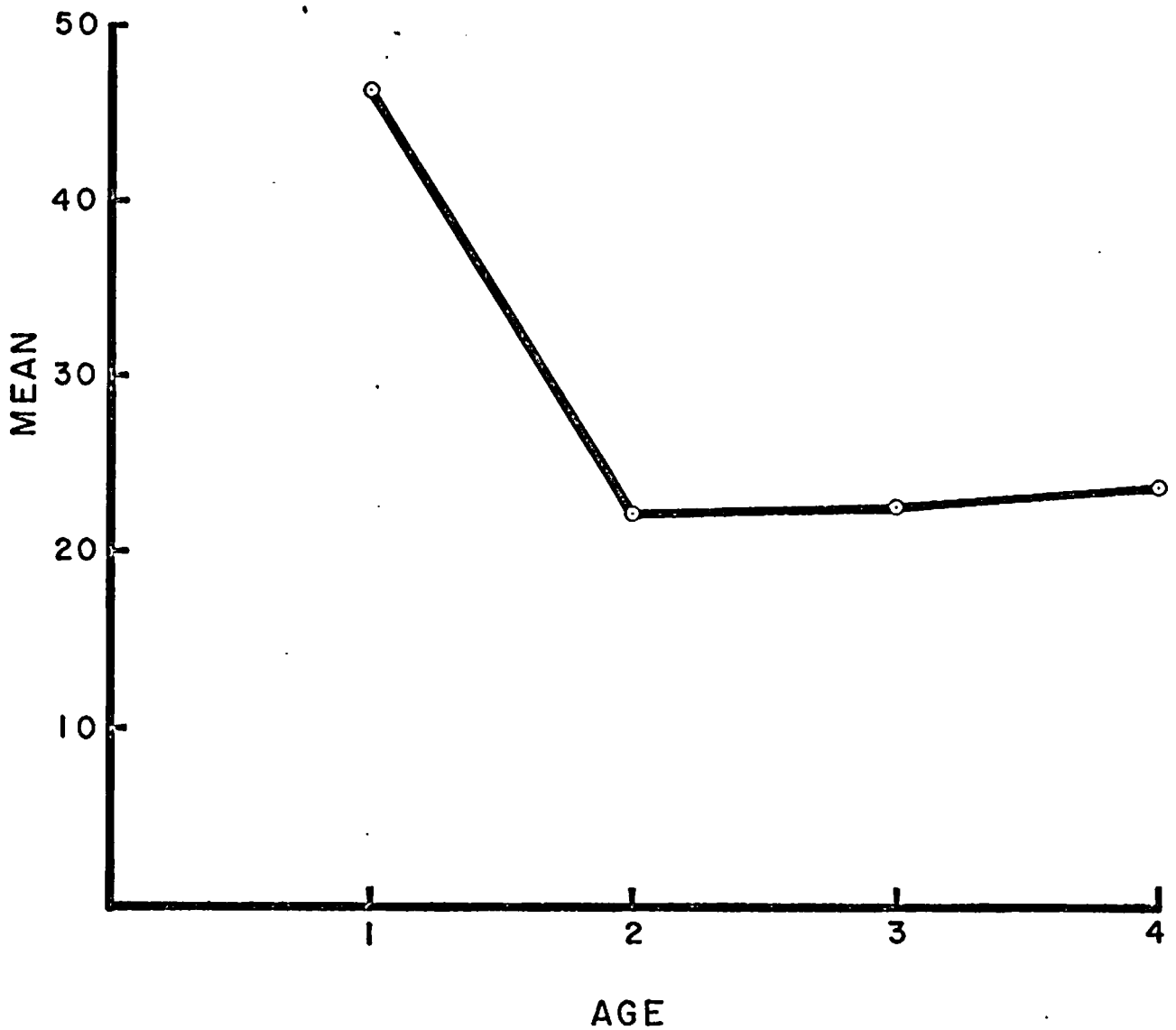


FIGURE 15

MEAN KILOHM BASAL SKIN RESISTANCE

FOR FOUR AGE LEVELS

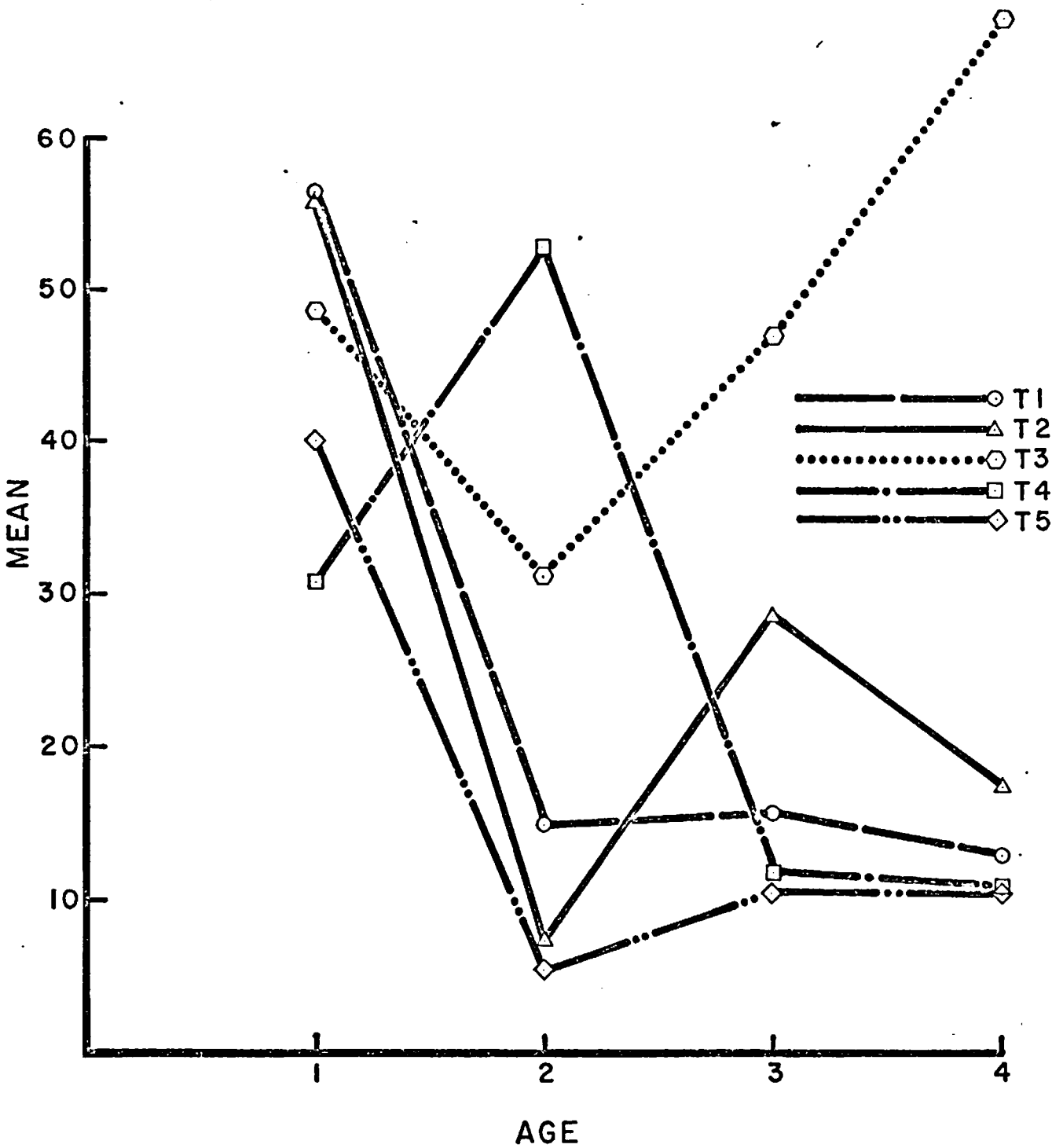


FIGURE 16

MEAN KILOHM BASAL SKIN RESISTANCE

FOR FIVE TREATMENT GROUPS AT FOUR

AGE LEVELS

TABLE 8

ANALYSIS OF VARIANCE:

GRAMS PRESSURE OF SPONTANEOUS LEG FLEXION

Source	df	MS	F Ratio
Treatment (T)	4	1409.623	19.00*
Age (A)	3	22151.744	298.66*
T X A	12	1024.040	13.80*
Subjects	180	74.170	
Order (O)	2	3.254	.85
T X O	8	9.469	2.40
A X O	6	3.295	.86
T X A X O	24	10.267	2.68*
S X O	360	3.816	

* p less than .01

of SLF for the various treatment groups is presented in Figure 17. These data for the age levels are presented in Figure 18, while the treatment by age data are displayed in Figure 19. These data strongly support the observation that dl PCL and I^{131} treated Ss are weaker than normal Ss.

Treatment comparisons of the current necessary to elicit minimum leg flexion did not yield significant differences (H = 6.26, p less than .10). Plots of treatment and age data are displayed in Figures 20 and 21. While there are no differences among the treatment groups, Figure 21 illustrates that the current necessary to elicit a minimum leg flexion for older Ss was greater than that required for Al Ss.

Analysis of the current necessary to elicit maximum leg flexion yielded significant treatment, age and treatment by age effects (Table 9). Figure 22 presents the mean current values for the five treatment groups. These data for the four age levels are presented in Figure 23. The age and treatment data are presented in Figure 24. These data indicate that from seven to 14 days the current necessary to elicit a maximum leg flexion increased sharply and remained stable thereafter. They also indicate that normal groups required a higher current value to elicit a maximum response than did dl PCL and I^{131} groups. The strikingly higher current value necessary to elicit the measure in pseudoconditioning groups at 14 days was not foreseen and is not amenable to explanation. The significant treatment and age interaction supports the advisability of establishing the UCS intensity empirically.

Comparison of the number of responses obtained to five presentations of twelve intensity levels of vibro-tactile stimulation is presented in

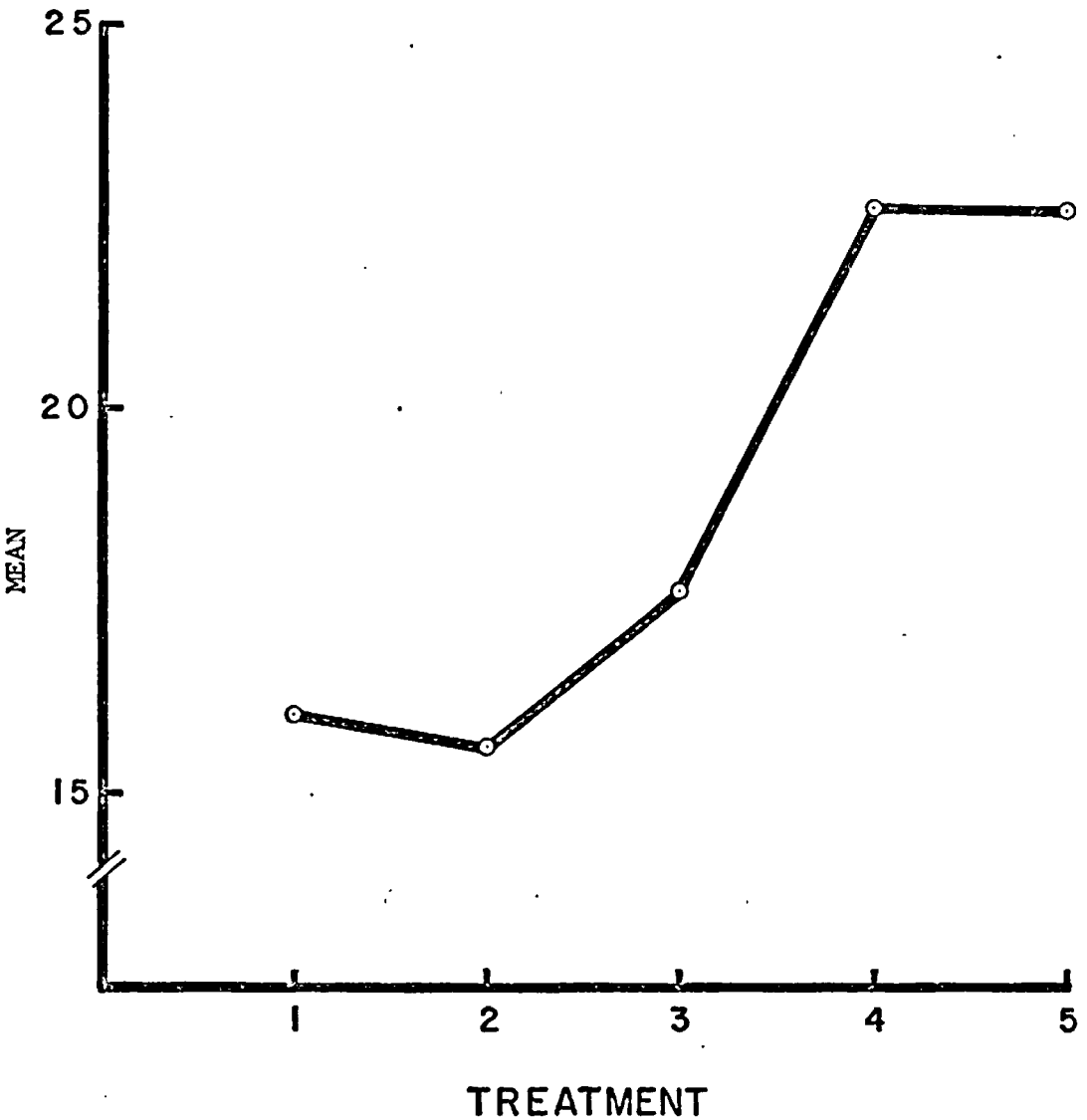


FIGURE 17

MEAN GRAMS PRESSURE OF SPONTANEOUS LEG FLEXION

FOR FIVE TREATMENT GROUPS

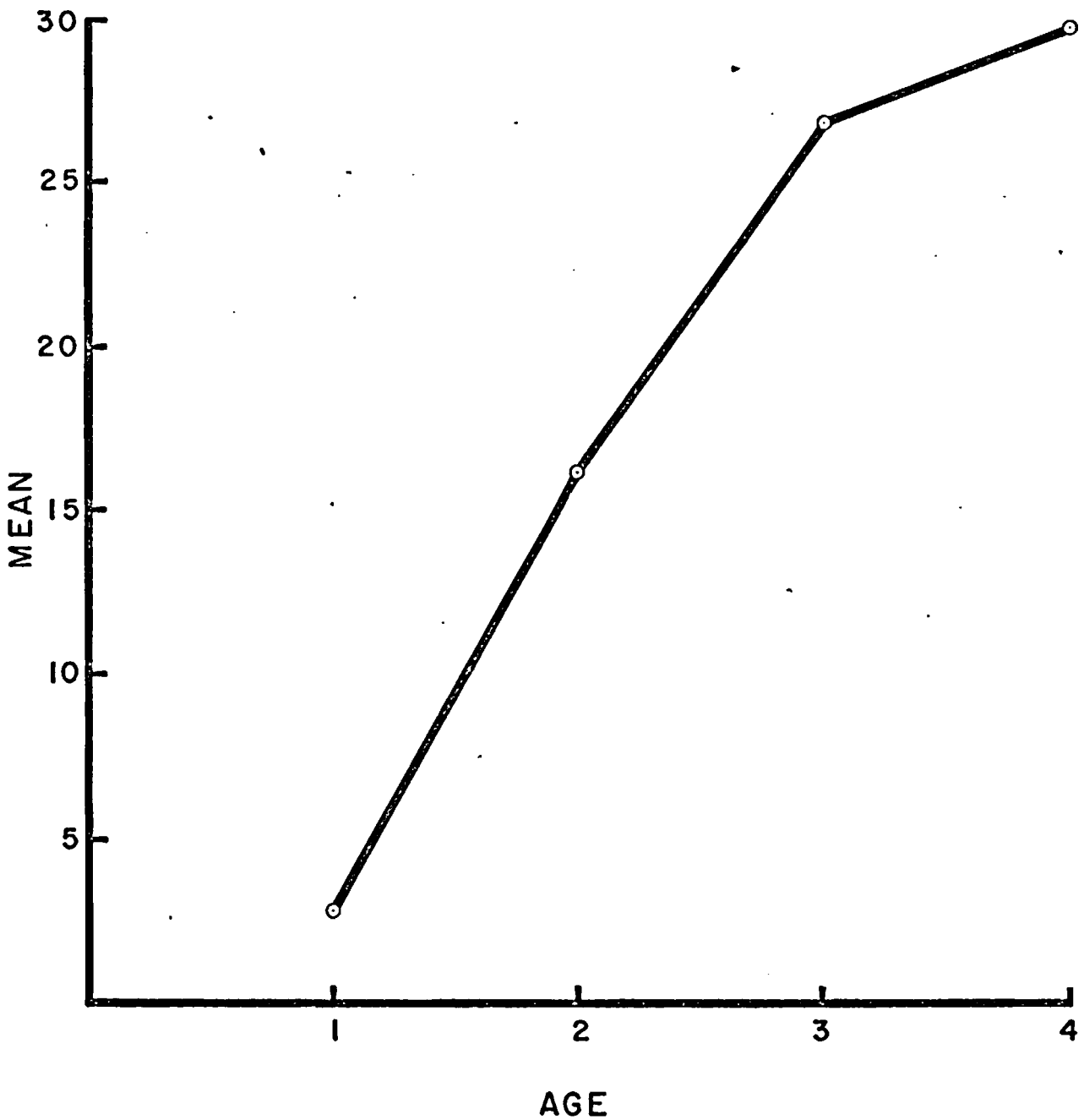


FIGURE 18

MEAN GRAMS PRESSURE OF SPONTANEOUS LEG FLEXION

FOR FOUR AGE LEVELS

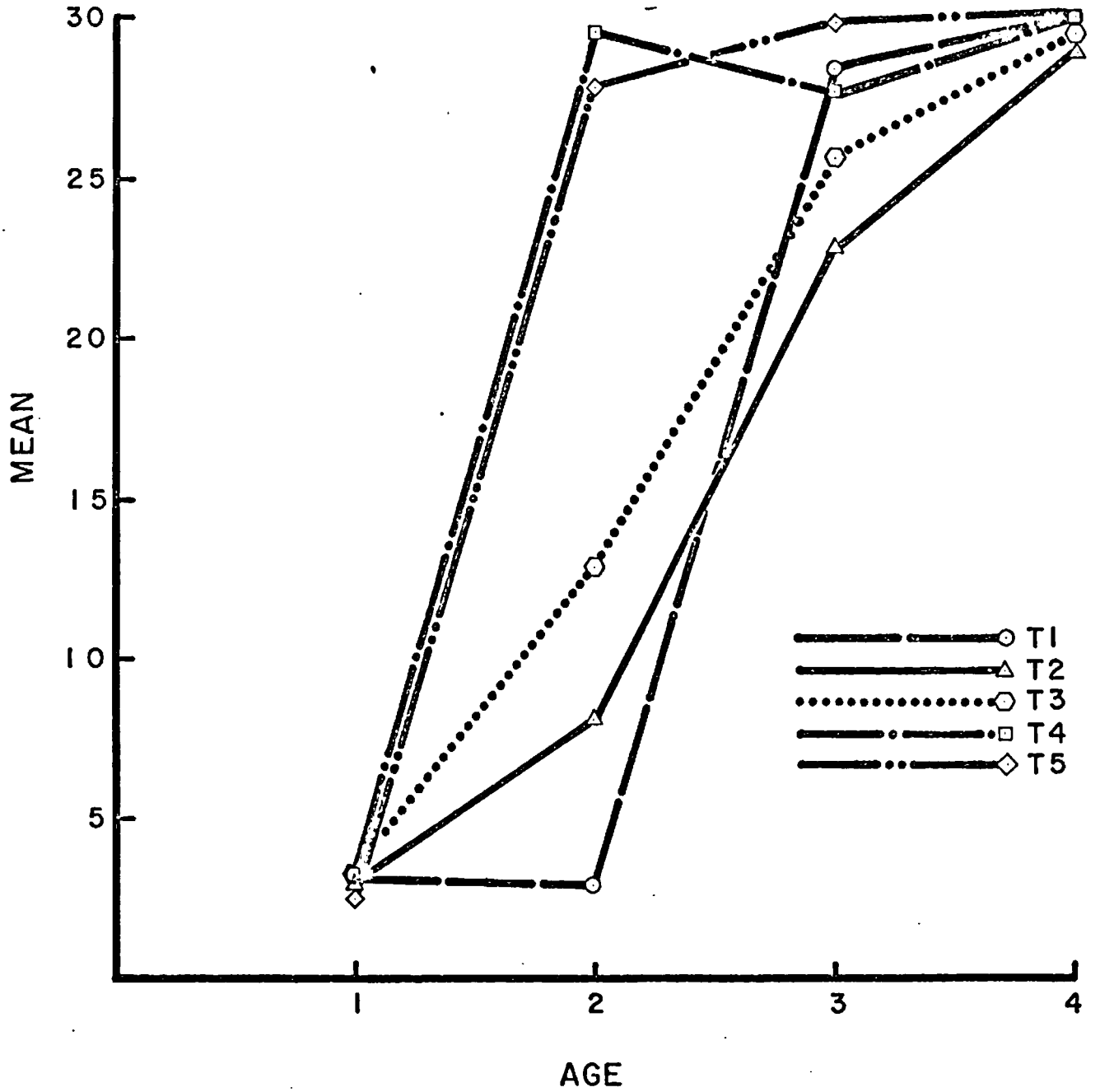


FIGURE 19

MEAN GRAMS PRESSURE OF SPONTANEOUS LEG FLEXION

FOR FIVE TREATMENT GROUPS AT FOUR AGE LEVELS

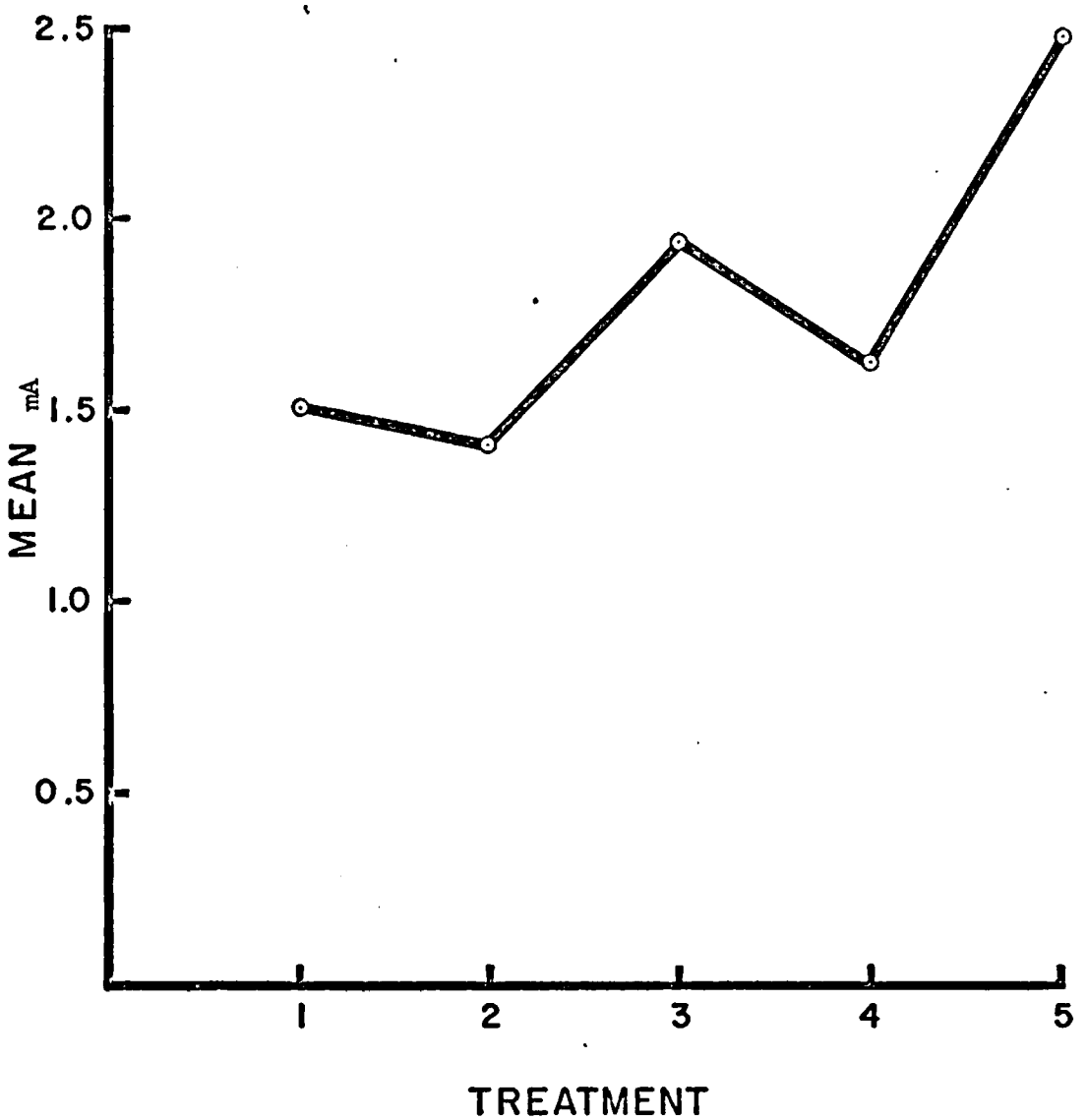


FIGURE 20

MEAN CURRENT NECESSARY TO ELICIT A MINIMUM LEG FLEXION

FOR FIVE TREATMENT GROUPS

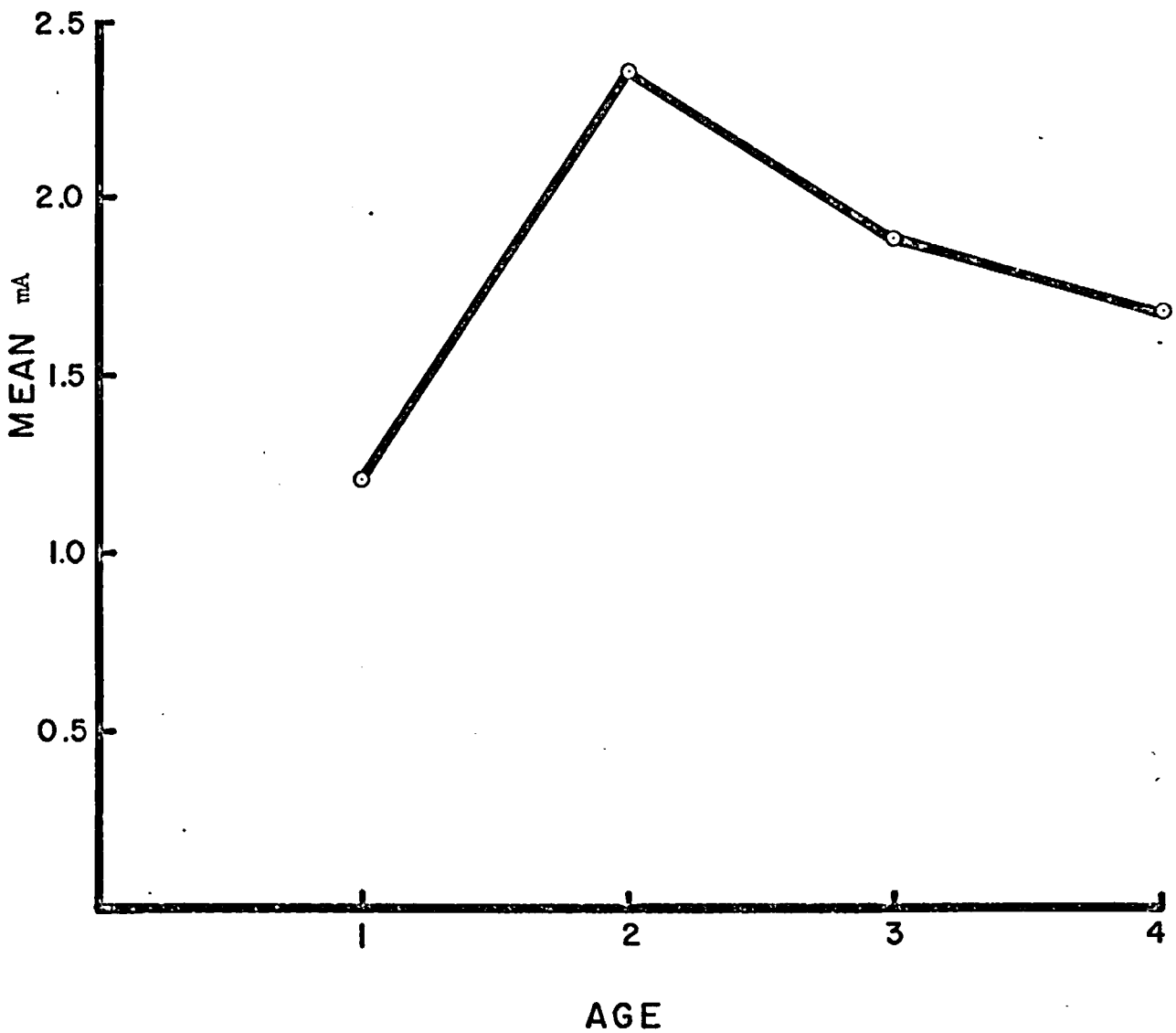


FIGURE 21

MEAN CURRENT NECESSARY TO ELICIT A MINIMUM LEG FLEXION

FOR FOUR AGE LEVELS

TABLE 9

ANALYSIS OF VARIANCE:

CURRENT TO ELICIT MAXIMUM LEG FLEXION

Source	df	MS	F Ratio
Treatment (T)	4	581.556	11.68*
Age (A)	3	809.938	16.28*
T X A	12	179.321	3.60*
Subjects	180	49.733	

* p less than .01

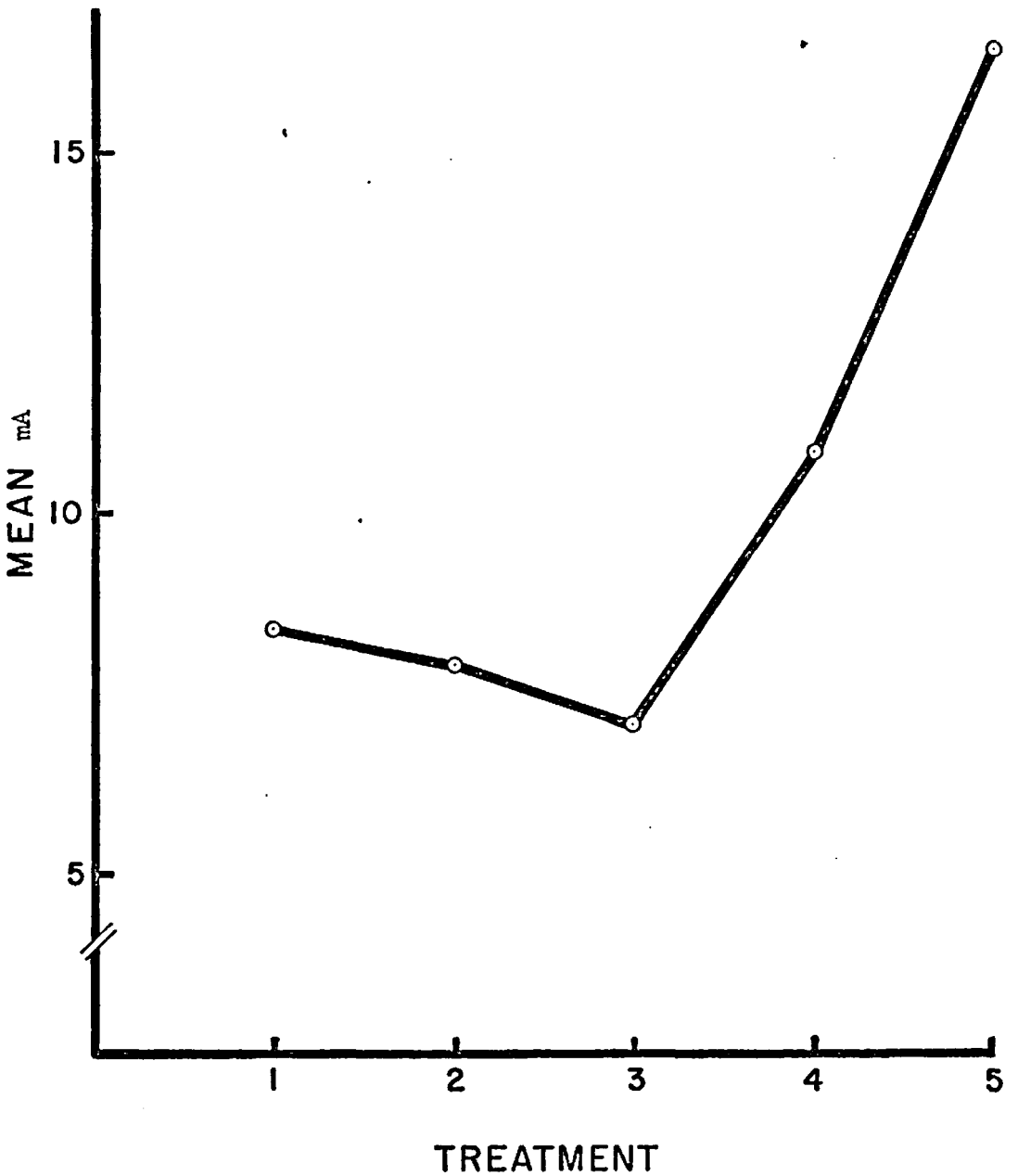


FIGURE 22

MEAN CURRENT NECESSARY TO ELICIT MAXIMUM LEG FLEXIONS

FOR FIVE TREATMENT GROUPS

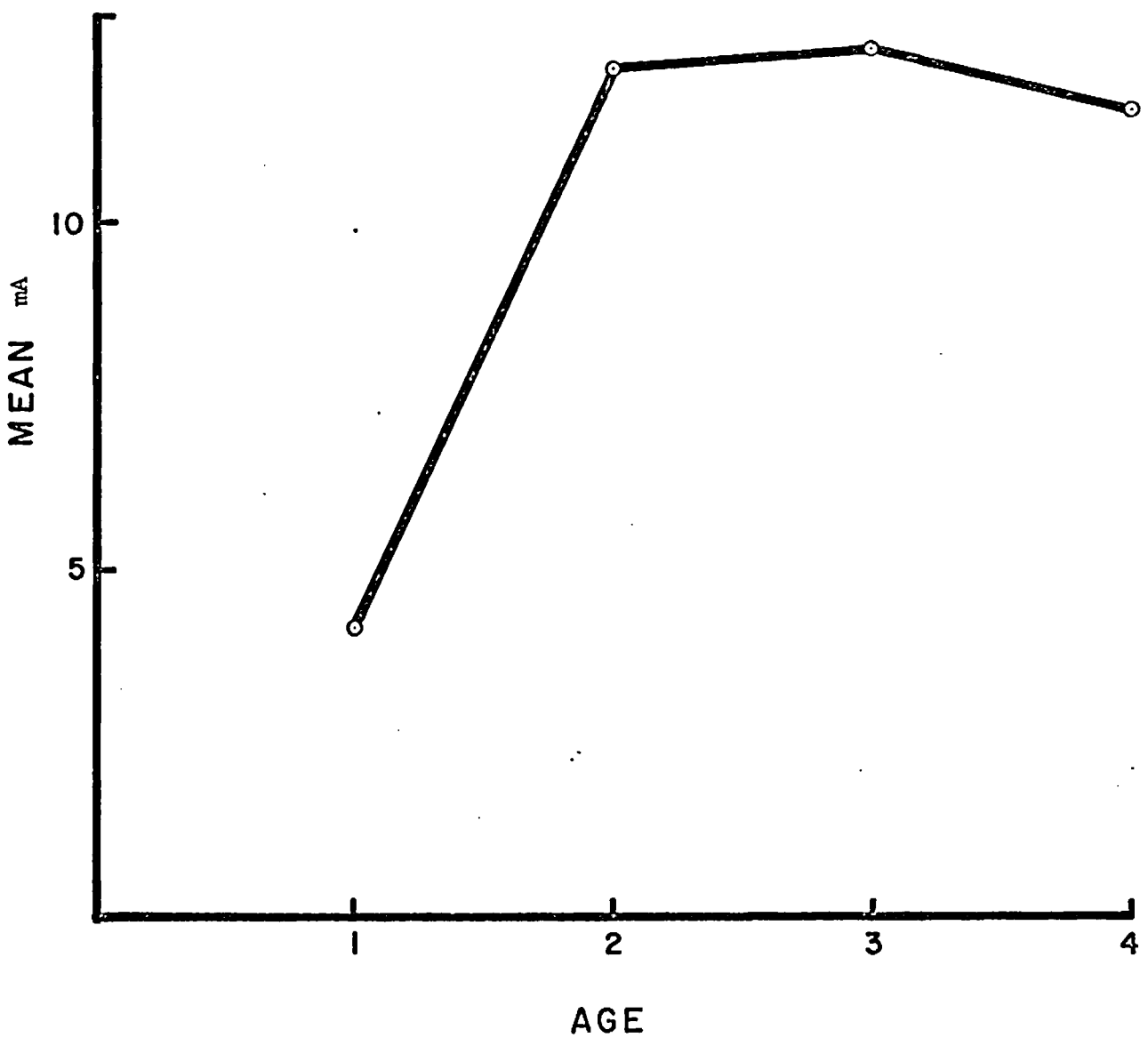


FIGURE 23

MEAN CURRENT NECESSARY TO ELICIT MAXIMUM LEG FLEXION

FOR FOUR AGE LEVELS

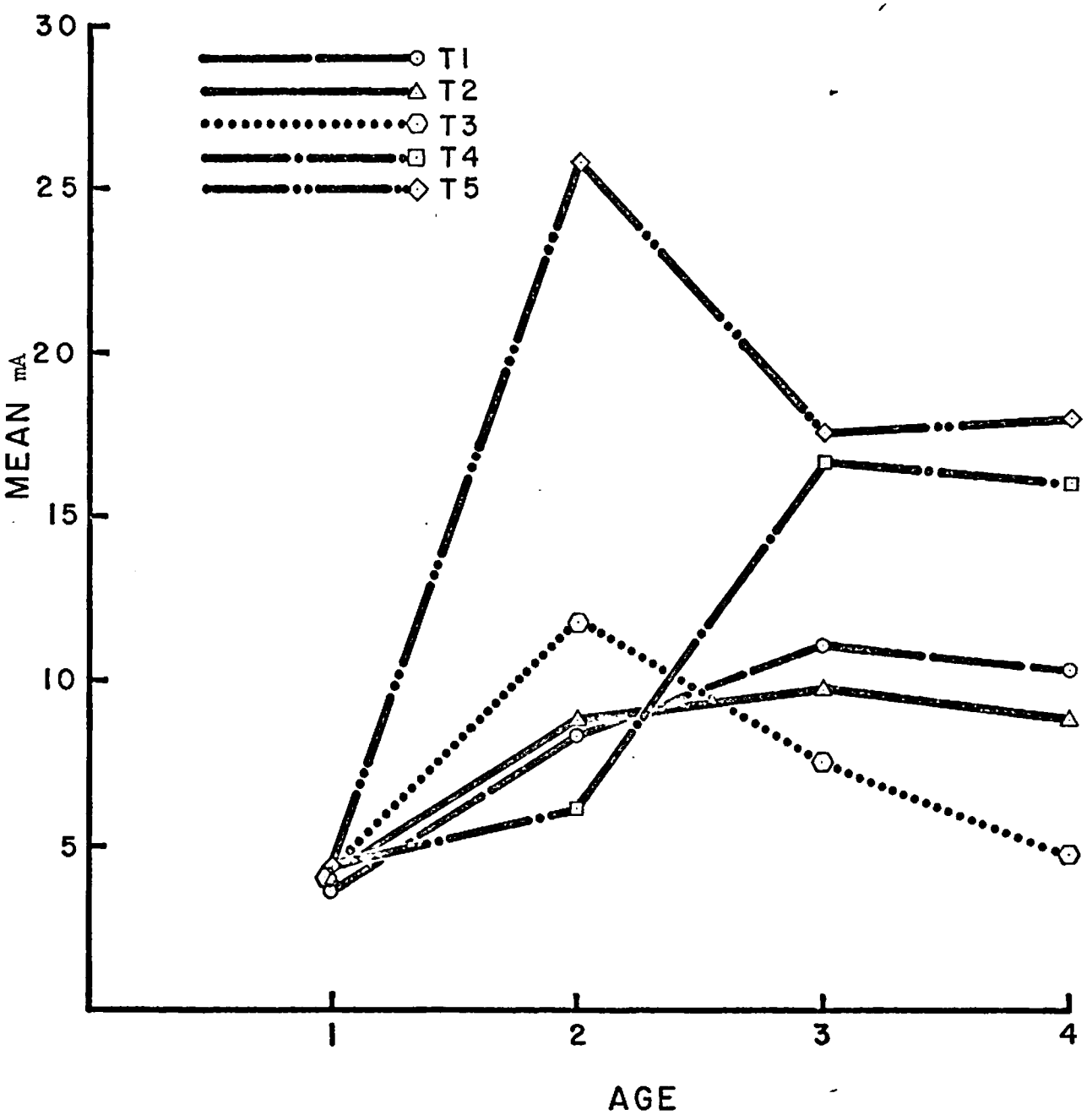


FIGURE 24

MEAN CURRENT NECESSARY TO ELICIT MAXIMUM LEG FLEXION

FOR FIVE TREATMENT GROUPS AT FOUR AGE LEVELS

Table 10. All possible comparisons are significant. The mean responses for the treatment groups are presented (Figure 25) as are the data for the four age levels (Figure 26). The mean responses for all Ss as a function of the 12 intensity levels are plotted in Figure 27. These data for the treatment groups are presented in Figure 28, while that of the four age levels are presented in Figure 29. The data indicate that the number of leg flexion responses increased as the intensity of vibro-tactile stimulation increased for all groups and the variety of interaction effects obtained support the need for establishing the CS level empirically.

Analysis of the number of trials necessary to reach an habituation criterion did not yield significant treatment effects ($H = 11.94$; p greater than .01) indicating that performance on this type of learning task did not differ among the groups.

As the current value used for the UCS was determined by the current necessary to elicit three successive full scale leg flexions, analysis of the UCS intensity data yielded differences paralleling those obtained for that data. Treatment, age and treatment by age effects were significant (Table 11). The mean UCS intensity used for the five conditioning groups is displayed in Figure 30. These data for the four age levels are shown in Figure 31, while Figure 32 shows the treatment by age relationship.

Analysis of the CS intensity level data yielded significant treatment, age and treatment by age effects (Table 12). The mean CS levels for the groups are presented in Figure 33. These data for the age levels are presented in Figure 34. The treatment by age relationship is presented

TABLE 10

ANALYSIS OF VARIANCE:

RESPONSES TO FIVE PRESENTATIONS OF

TWELVE INTENSITY LEVELS OF VIBRO-TACTILE STIMULATION

Source	df	MS	F Ratio
Treatment (T)	4	29.917	3.90*
Age (A)	3	134.612	17.55*
T X A	12	29.100	3.79*
Subjects	180	7.669	
Vibro-Tactile Level (V)	11	45.903	54.86*
T X V	44	1.395	1.66*
A X V	33	2.402	2.87*
T X A X V	132	1.348	1.61*
S X V	1980	0.836	

* p less than .01

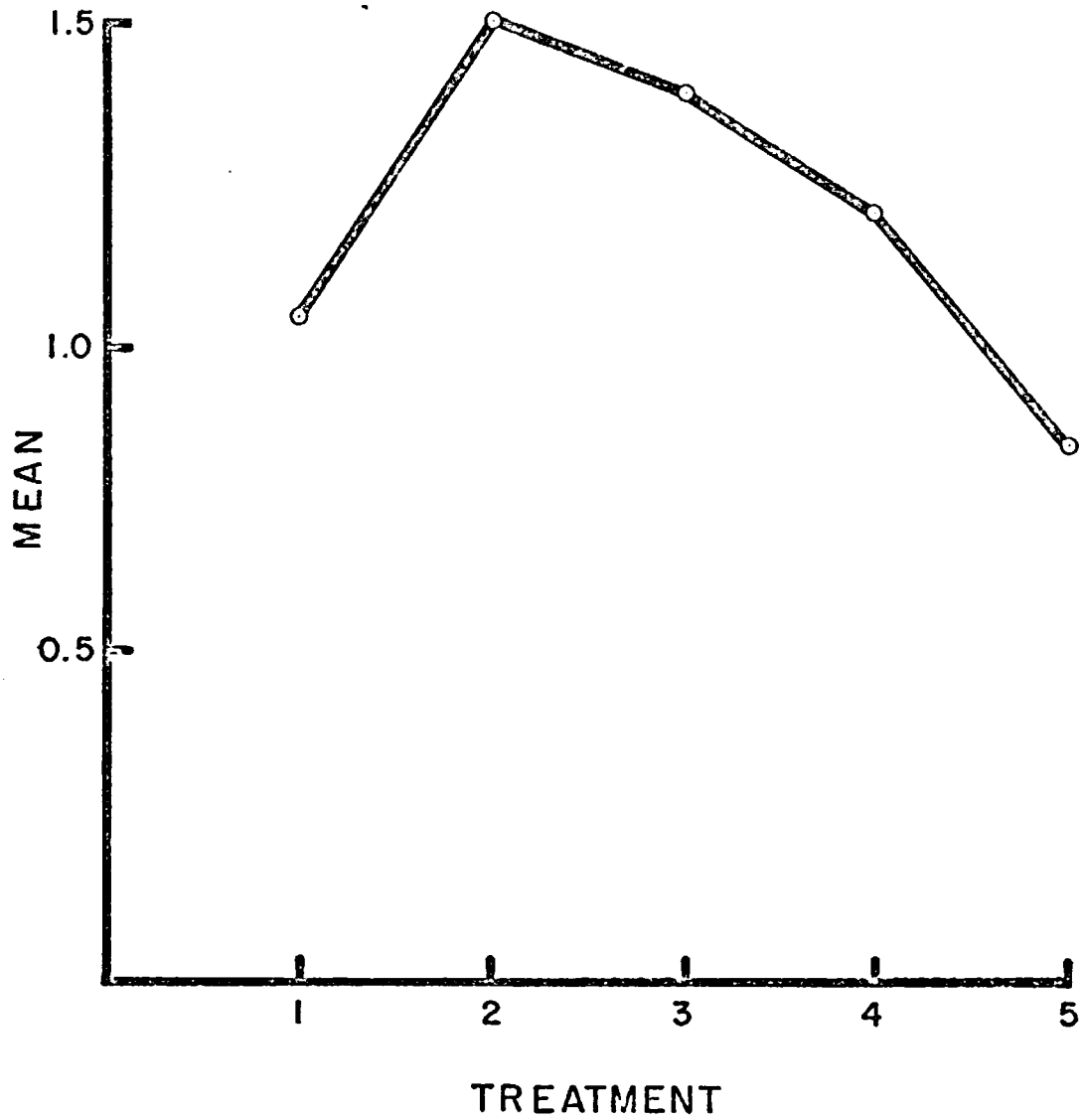


FIGURE 25

MEAN RESPONSES TO VIBRO-TACTILE STIMULATION

FOR FIVE TREATMENT GROUPS

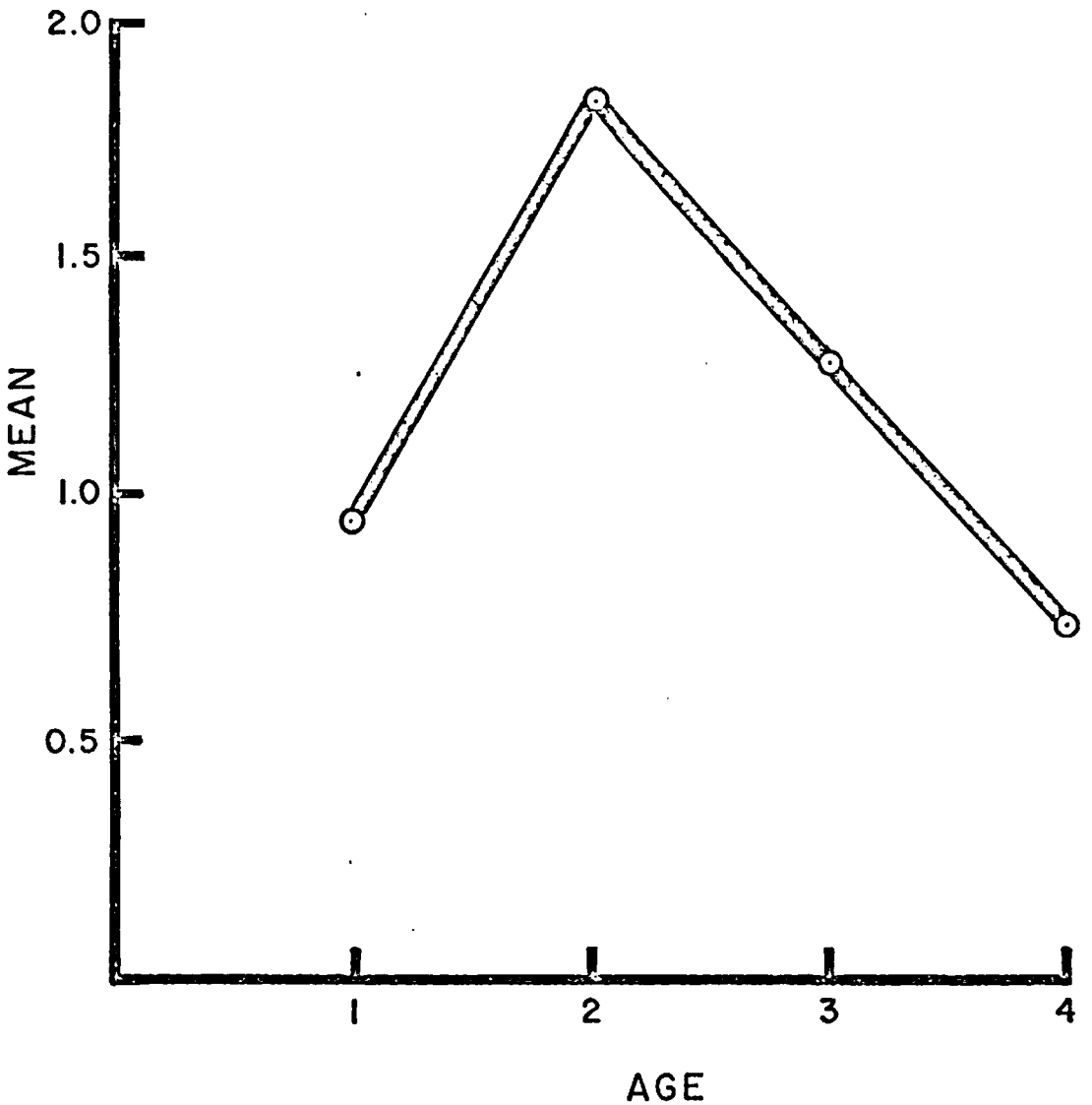


FIGURE 26

MEAN RESPONSES TO VIBRO-TACTILE STIMULATION

FOR FOUR AGE LEVELS

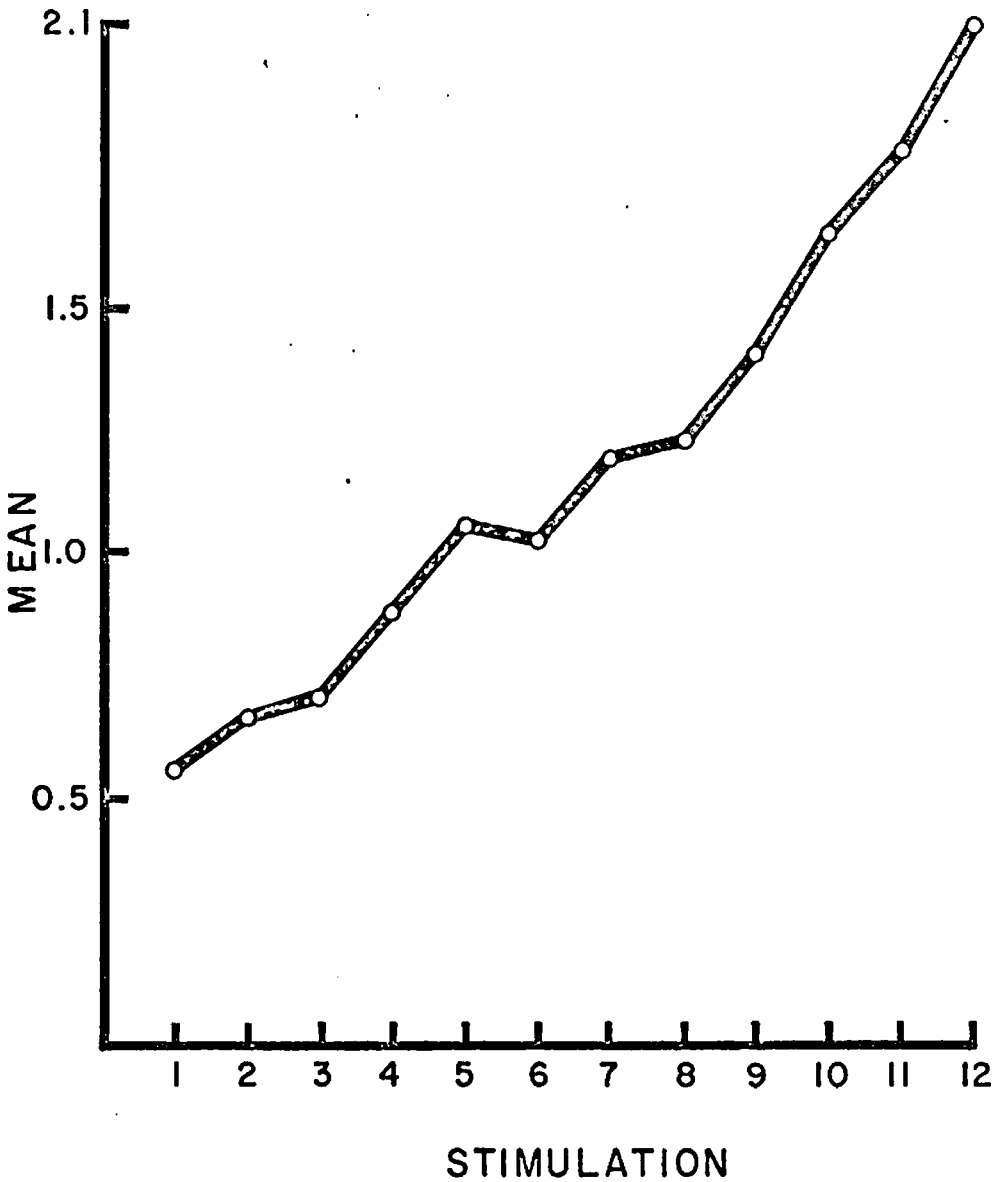


FIGURE 27

MEAN RESPONSES TO TWELVE LEVELS

OF VIBRO-TACTILE STIMULATION

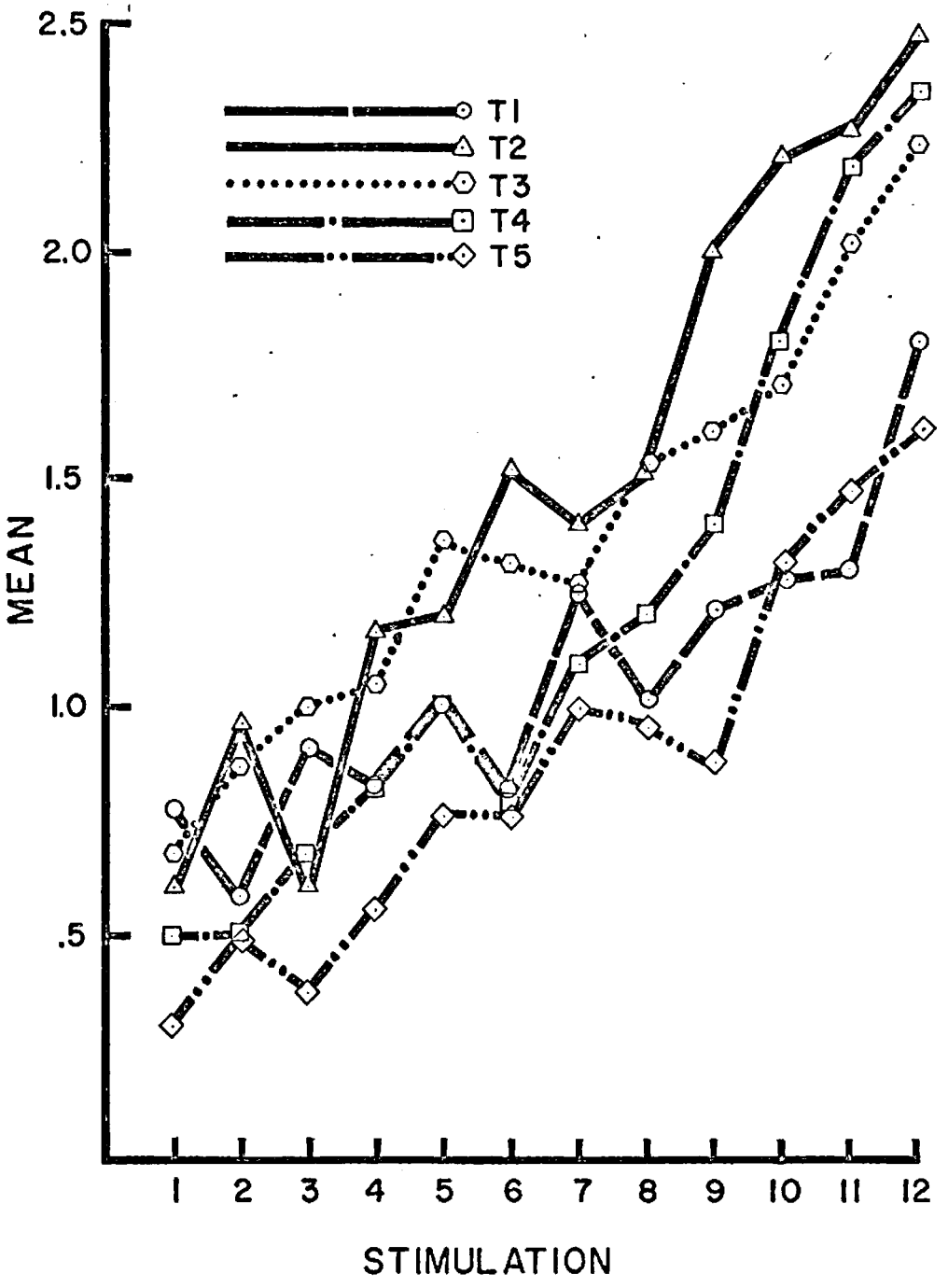


FIGURE 28

MEAN RESPONSES TO TWELVE LEVELS OF VIBRO-TACTILE STIMULATION FOR FIVE TREATMENT GROUPS

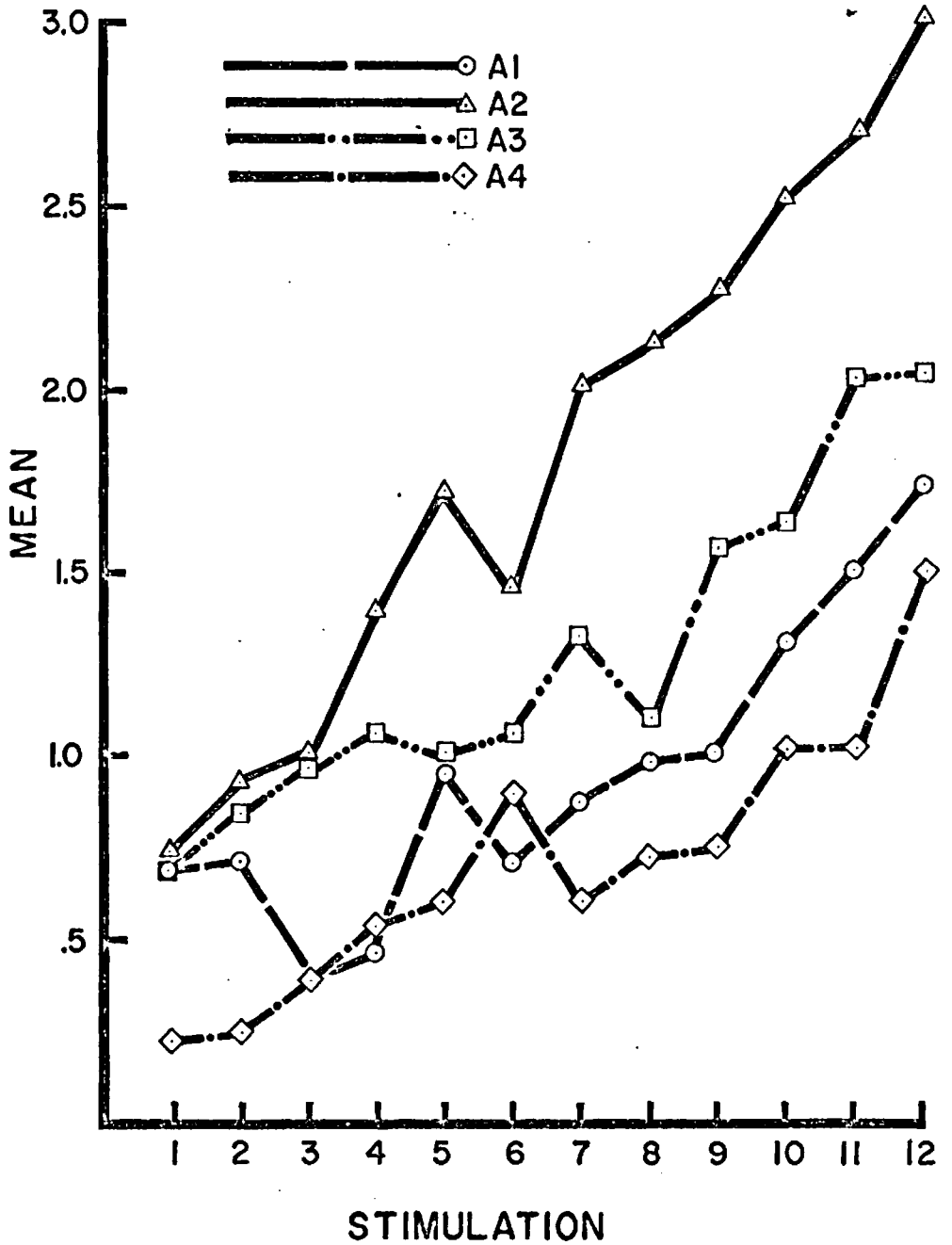


FIGURE 29

MEAN RESPONSES TO TWELVE LEVELS OF VIBRO-TACTILE STIMULATION FOR FOUR AGE LEVELS

TABLE 11

ANALYSIS OF VARIANCE:

UNCONDITIONED STIMULUS LEVEL

Source	df	MS	F Ratio
Treatment (T)	4	578.939	12.19*
Age (A)	3	981.341	20.67*
T X A	12	226.483	4.77*
Subjects	180	47.466	

* p less than .01

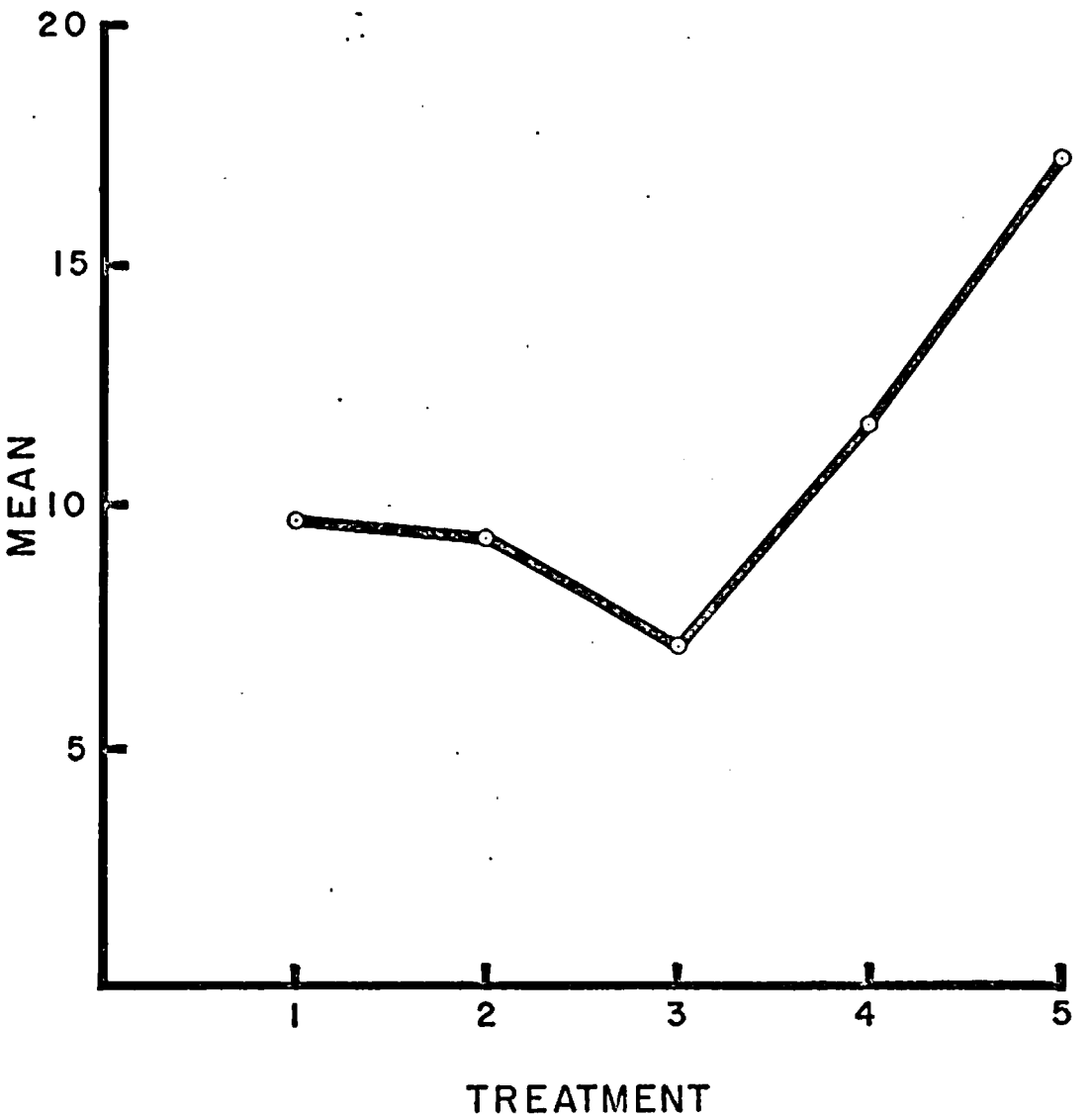
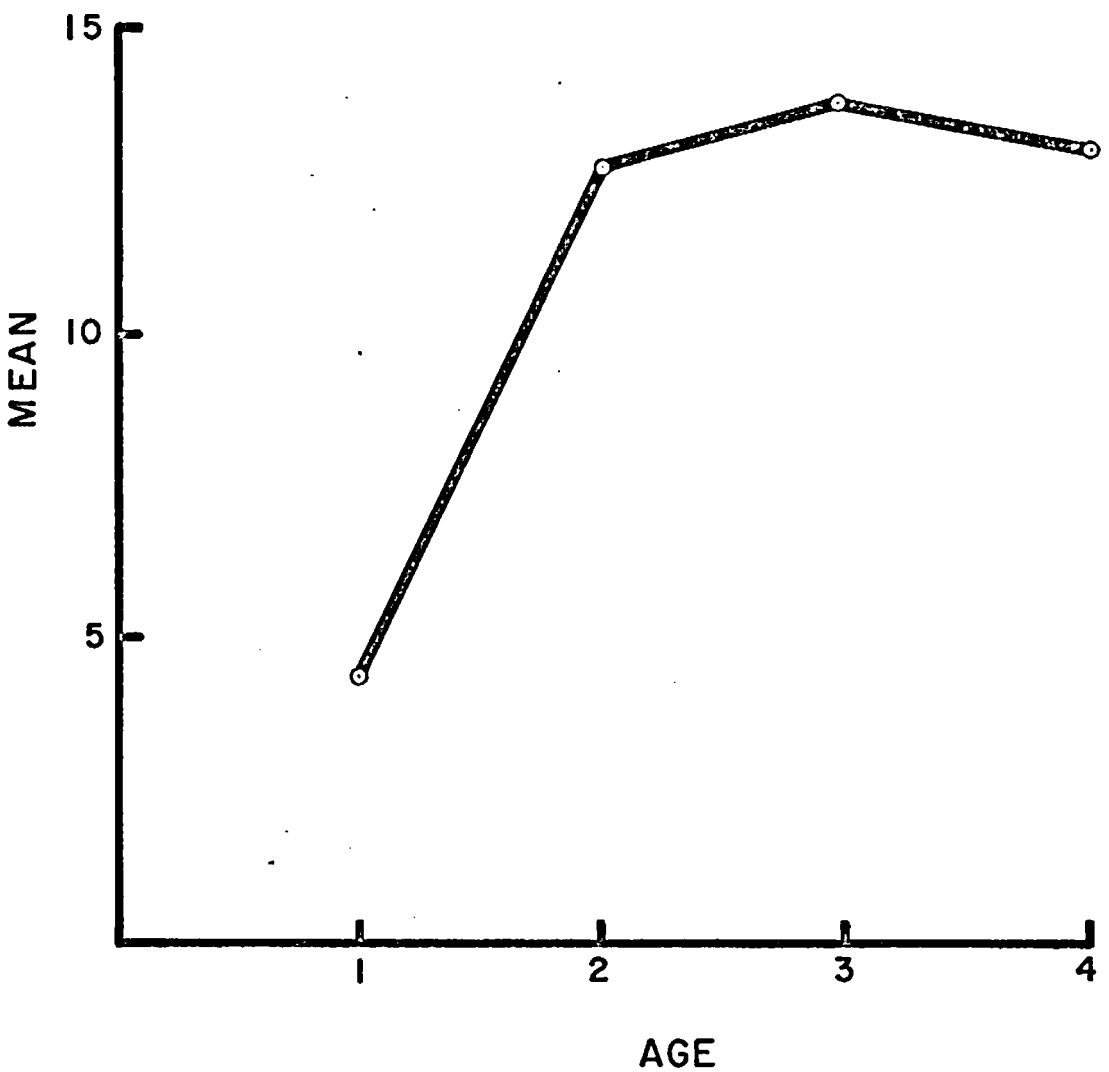


FIGURE 30

MEAN UNCONDITIONED STIMULUS LEVEL

FOR FIVE TREATMENT GROUPS



MEAN UNCONDITIONED STIMULUS LEVEL

FOR FOUR AGE LEVELS

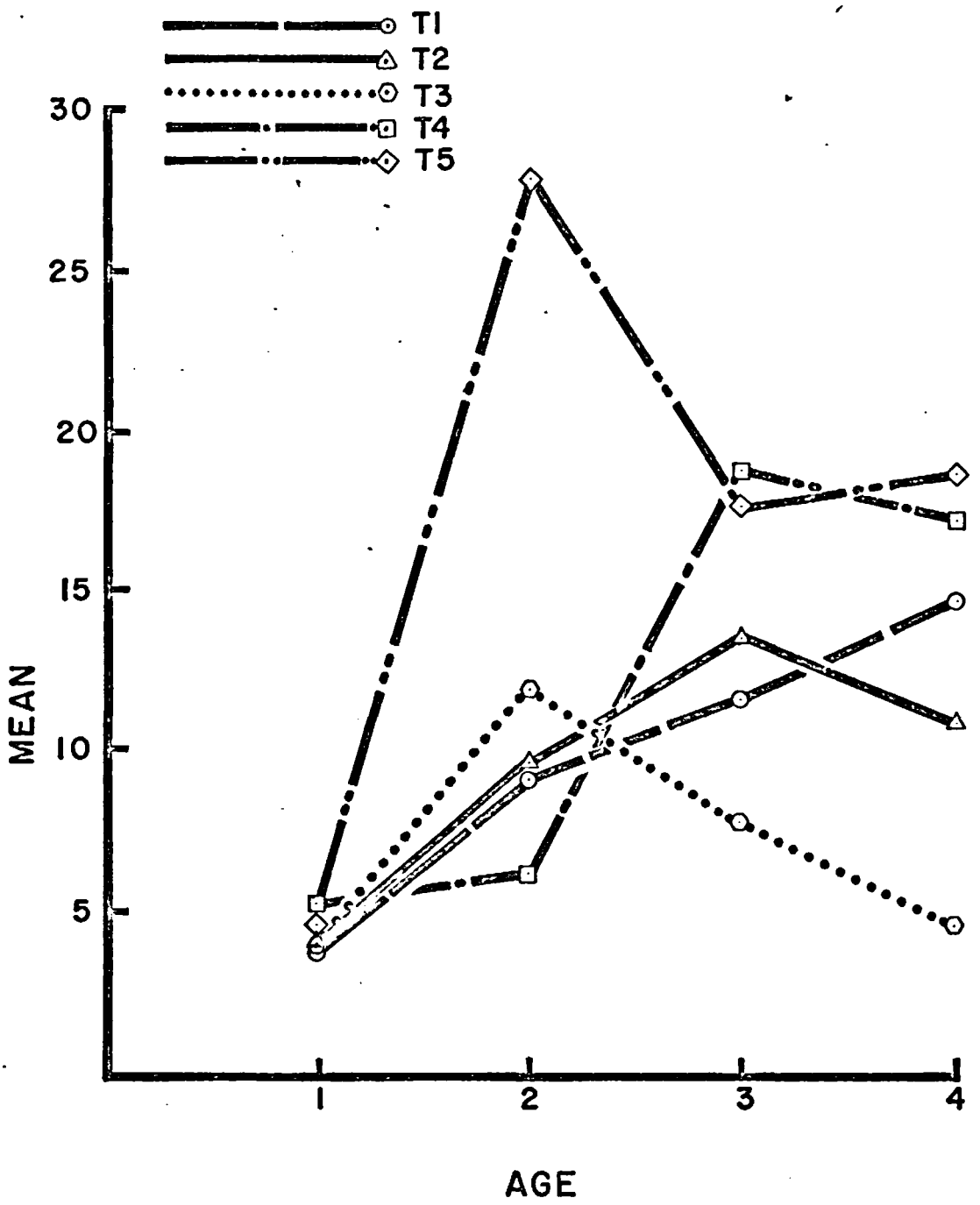


FIGURE 32

MEAN UNCONDITIONED STIMULUS LEVEL

FOR FIVE TREATMENT GROUPS AT FOUR AGE LEVELS

TABLE 12

ANALYSIS OF VARIANCE:

CONDITIONED STIMULUS INTENSITY

Source	df	MS	F Ratio
Treatment (T)	4	2.410	4.74*
Age (A)	3	9.101	17.93*
T X A	12	2.863	5.64*
Subjects	180	0.507	

* p less than .01

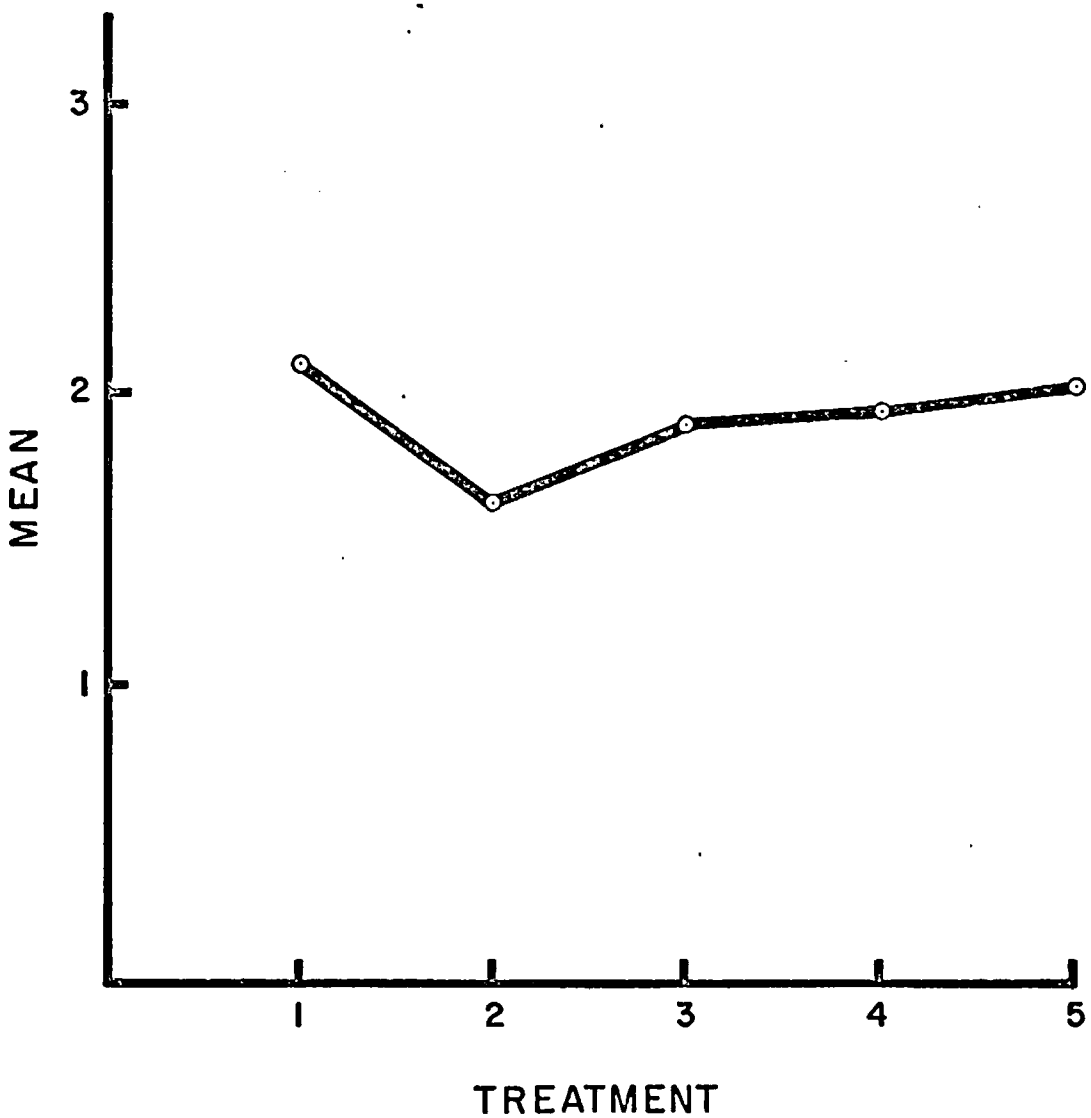


FIGURE 33

MEAN CONDITIONED STIMULUS LEVEL

FOR FIVE TREATMENT GROUPS

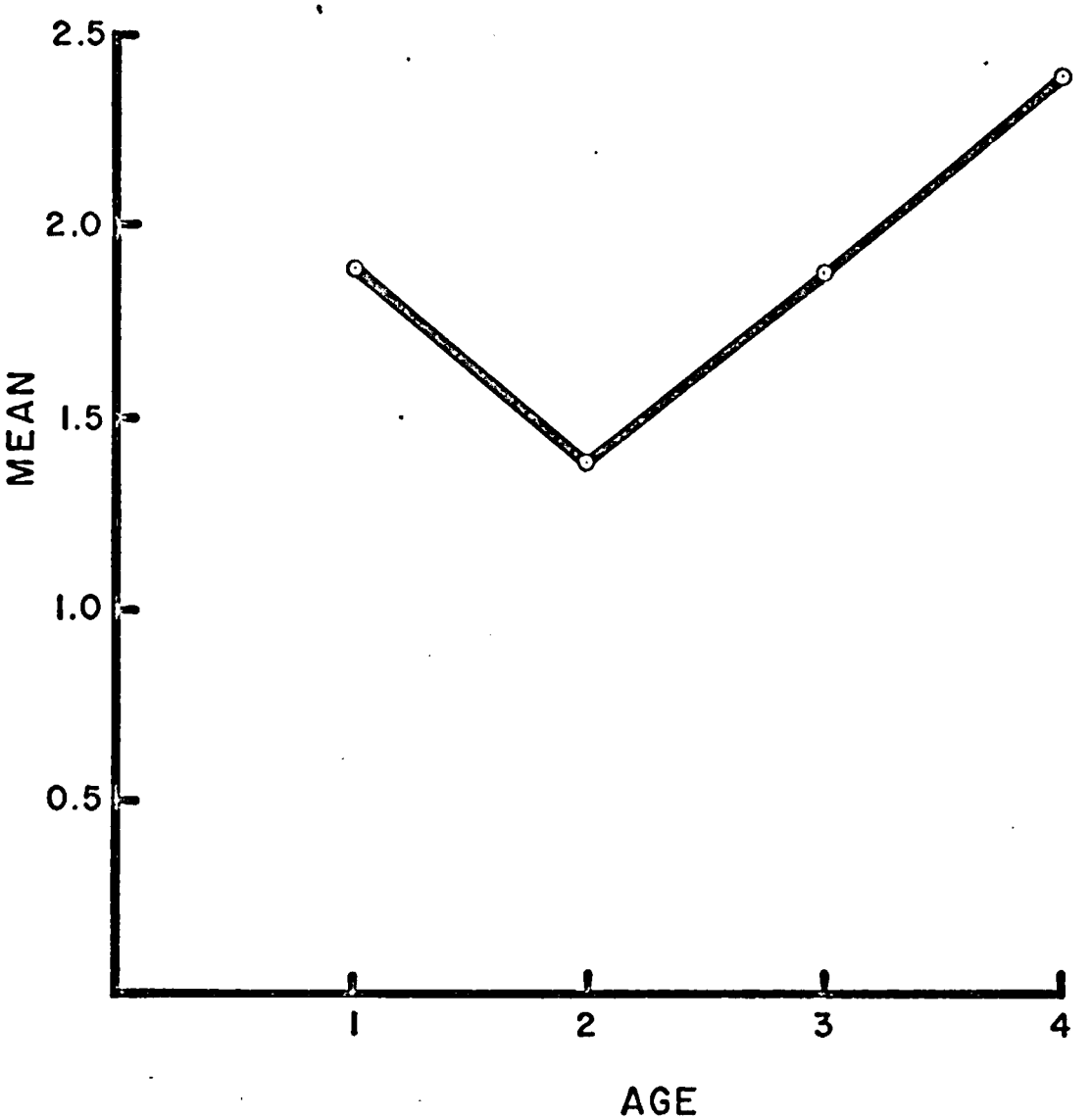


FIGURE 34

MEAN CONDITIONED STIMULUS LEVEL

FOR FOUR AGE LEVELS

in Figure 35. These data indicate that at younger ages dl PCL treated Ss required CS intensities lower than those of normal Ss while I^{131} Ss required levels higher than those of normals. At older ages, dl PCL (low dosage) and I^{131} treated Ss required CS intensities lower than those of normal Ss while the dl PCL (high dosage) Ss required levels above those of normals.

Histological examination of serial slides of thyroid tissue obtained from 14 T3 Ss and 14 T4 Ss revealed that the thyroid of the T3 Ss was clearly nonfunctional while that of T4 S was normal. These data indicate that the I^{131} treatment effectively induced a cretin condition in T3 groups.

Analysis of the μg/g brain 5-HT data for the groups yielded significant treatment, age and treatment by age effects (Table 13). Individual comparisons showed that T5, T8, T3 and T4 groups had significantly higher brain 5-HT levels than T1, T2, T6 and T7 groups (Table 14). In addition, T4 groups had significantly higher brain 5-HT levels than T5 groups. Individual comparisons of T4 groups 5-HT levels for the four ages showed those of A4 Ss to be significantly higher than A1 or A3 Ss, while they did not differ from those of A2 Ss (Table 15). A comparison of T8 Ss at four age levels showed that brain 5-HT levels at A4 were significantly higher than at A1, A2, or A3 (Table 16). Figure 36 shows the mean μg/g brain 5-HT levels for the eight treatment groups. These data for the four age levels are presented in Figure 37 and Figure 38 (a and b) shows the treatment and age relationship. These data strongly support the hypothesis that dl PCL treatment reduces brain 5-HT, and, thus, they support the hypothesis that dl PCL treatment would produce biochemical

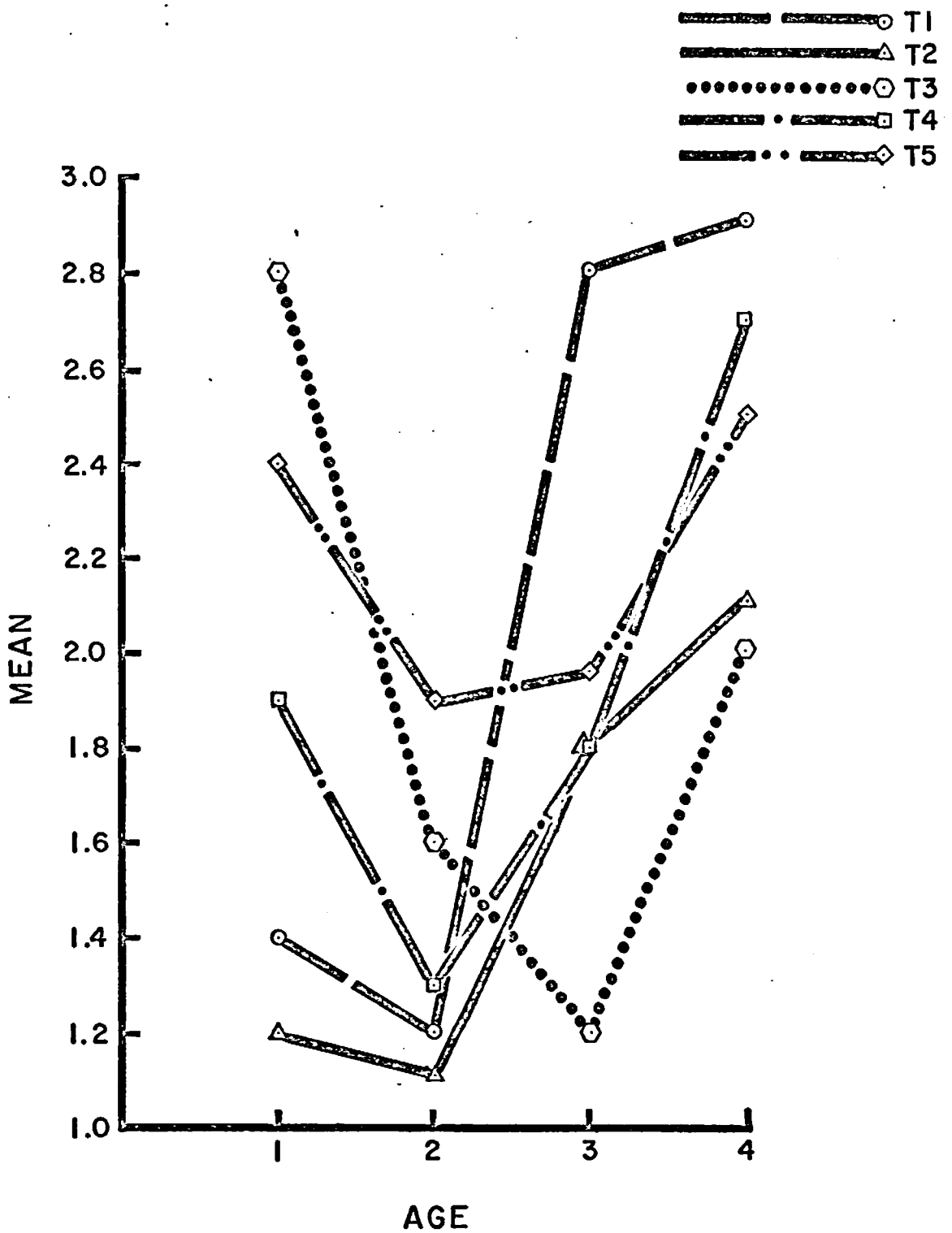


FIGURE 35

MEAN CONDITIONED STIMULUS LEVEL

FOR FIVE TREATMENT GROUPS AT FOUR AGE LEVELS

TABLE 13

ANALYSIS OF VARIANCE:

BRAIN SEROTONIN (μg/g)

Source	df	MS	F Ratio
Treatment (T)	7	0.15768	78.36*
Age (A)	3	0.10010	49.75*
T X A	21	0.01236	6.14*
Subjects	170	0.00201	

* p less than .01

TABLE 14

TUKEY (a) TEST OF TREATMENT MEANS

BRAIN SEROTONIN (μg/g)

Treatment	T6	T2	T1	T7	T5	T8	T3	T4
T6 (PCL-HC)					*	*	*	*
T2 (PCL-LE)					*	*	*	*
T1 (PCL-HE)					*	*	*	*
T7 (PCL-LC)					*	*	*	*
T5 (Pseudo)								*
T8 (Nor Cont)								
T3 (I^{131})								
T4 (Nor Cond)								

* p less than .01

TABLE 15

TUKEY (a) TEST OF AGE MEANS

T4 BRAIN SEROTONIN (μg/g)

Age	A1	A3	A2	A4
A1 (7 days)				*
A3 (21 days)				*
A2 (14 days)				
A4 (28 days)				

* p less than .01

TABLE 16

TUKEY (a) TEST OF AGE MEANS

T8 BRAIN SEROTONIN (μg/g)

Age	A1	A2	A3	A4
A1 (7 days)				*
A2 (14 days)				*
A3 (21 days)				*
A4 (28 days)				

* p less than .01

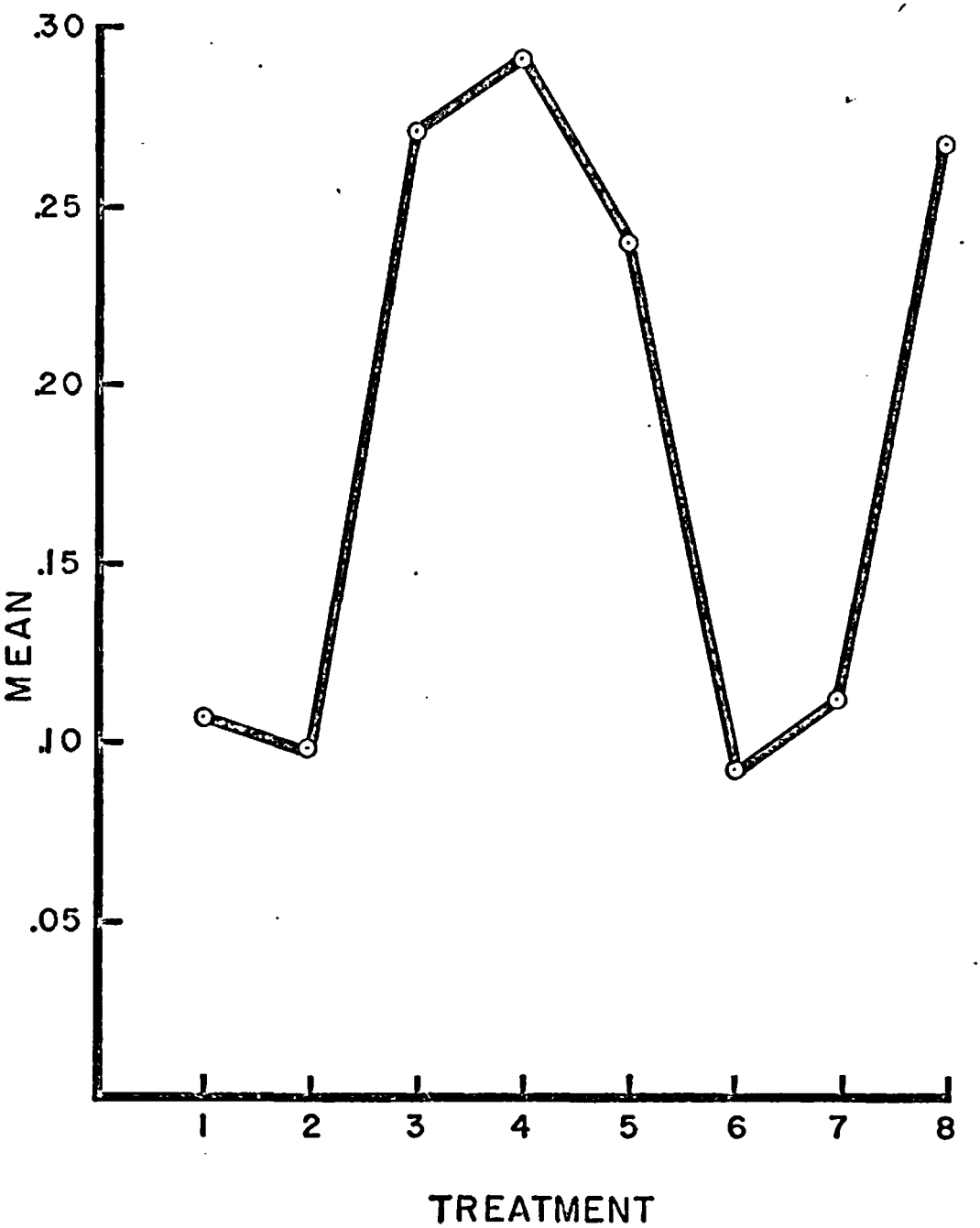


FIGURE 36

MEAN µg/g BRAIN SEROTONIN

FOR EIGHT TREATMENT GROUPS

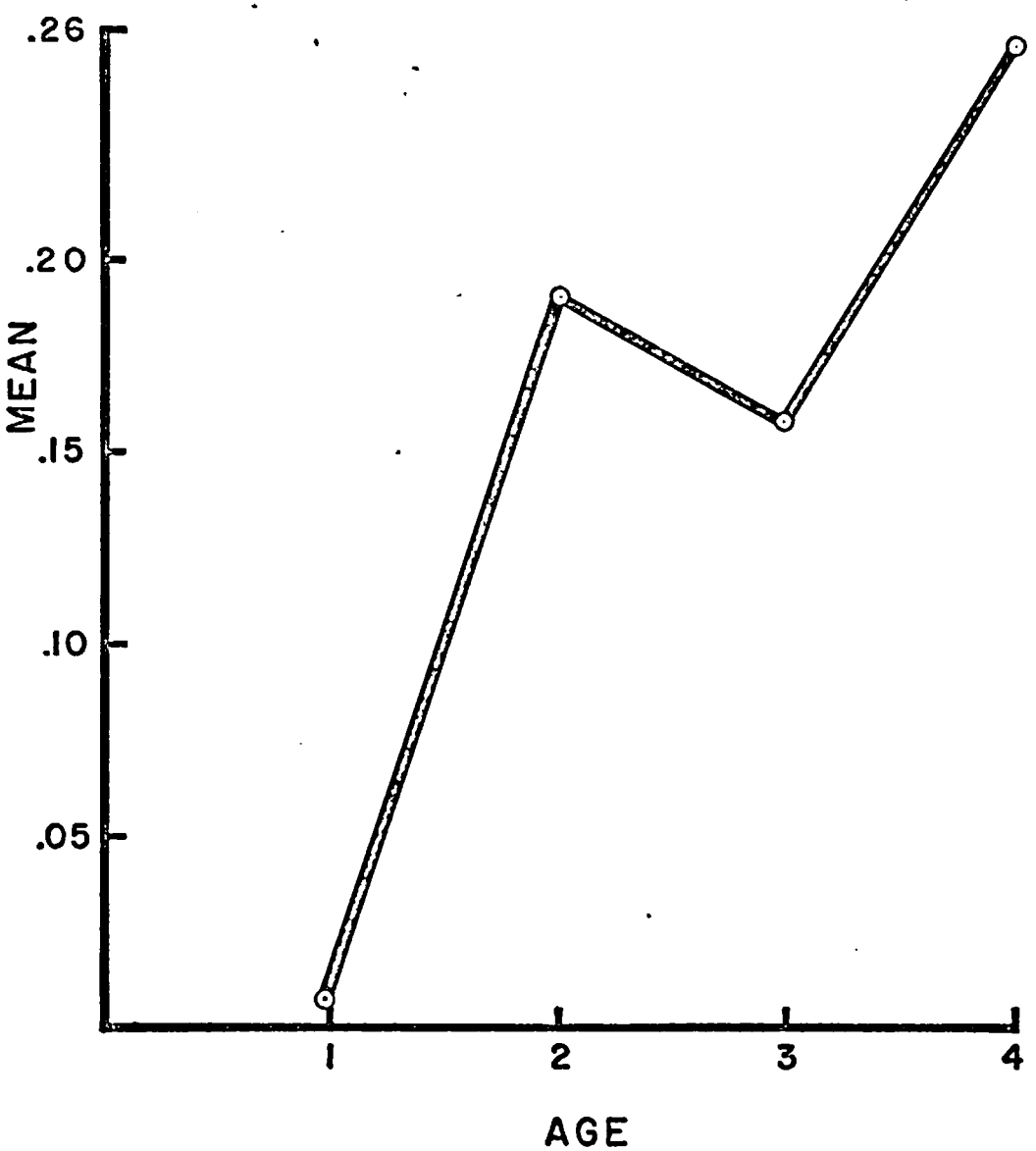


FIGURE 37

MEAN μg/g BRAIN SEROTONIN

FOR FOUR AGE LEVELS

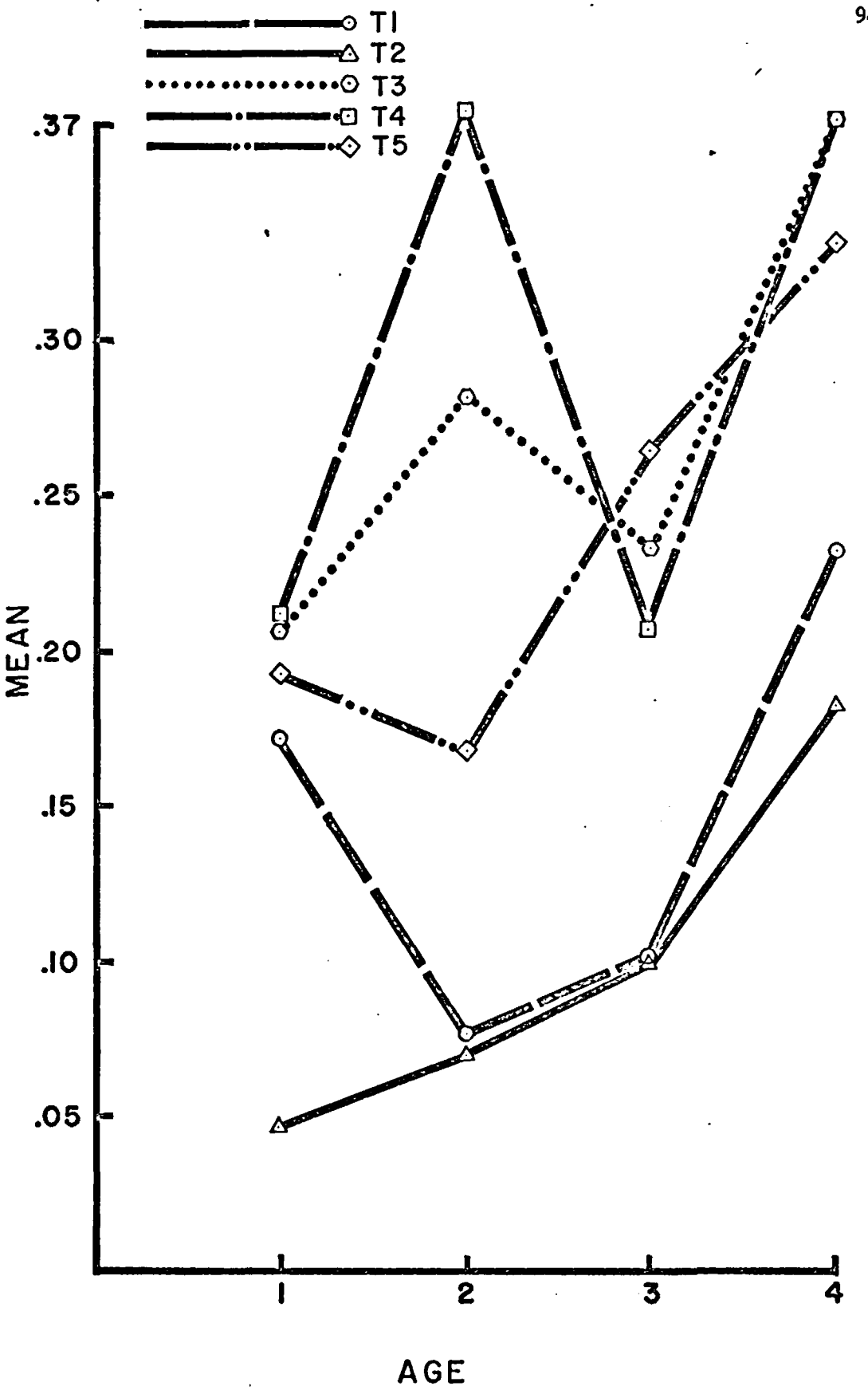


FIGURE 38a

MEAN μg/g BRAIN SEROTONIN
FOR FIVE CONDITIONING GROUPS AT FOUR AGE LEVELS

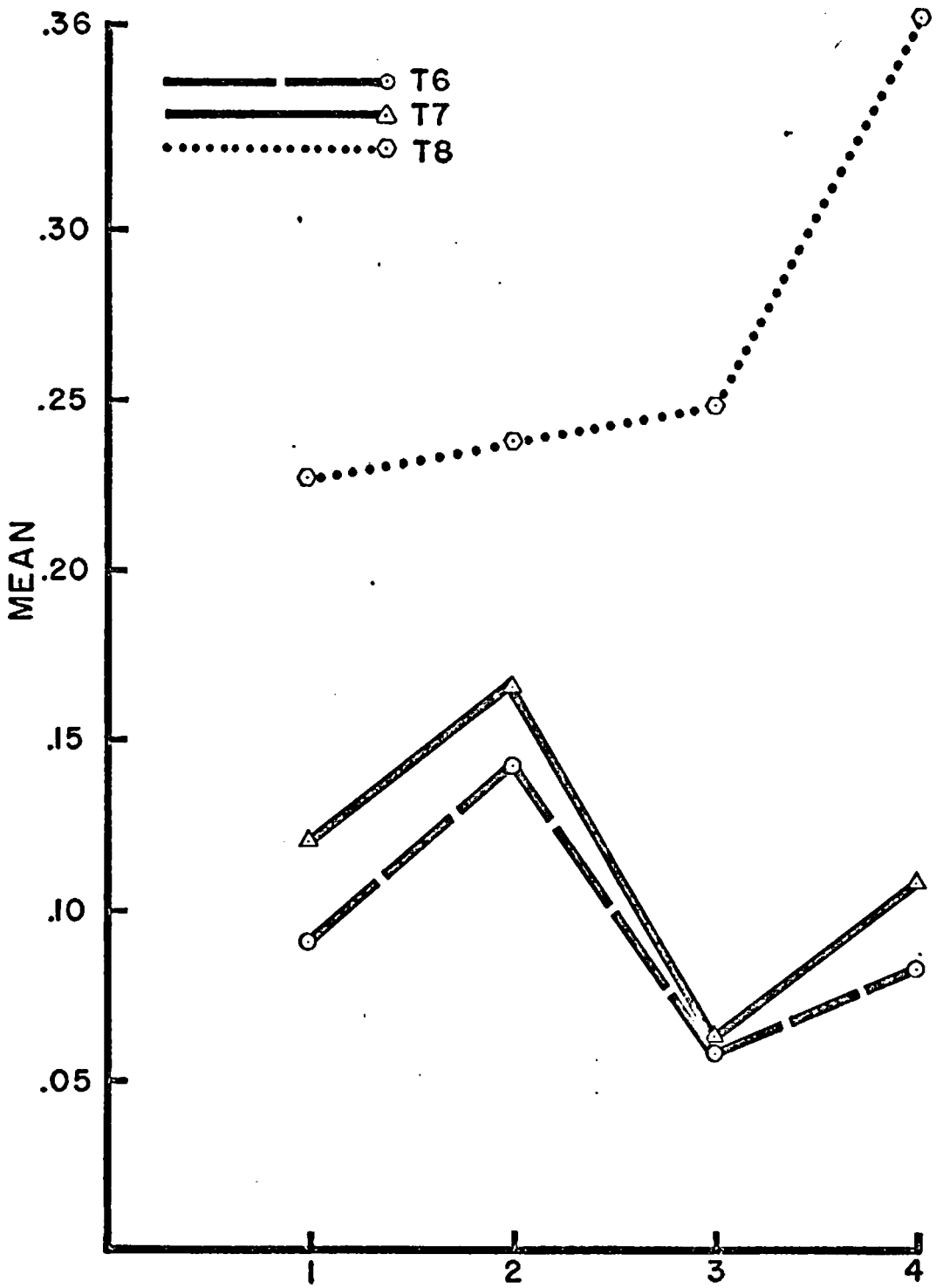


FIGURE 38b

MEAN µg/g BRAIN SEROTONIN
FOR TWO DRUG CONTROL GROUPS
AND A NORMAL CONTROL GROUP
AT FOUR AGE LEVELS

characteristics of PKU. They also indicate that brain 5-HT levels increased as a function of age for the normal control group.

Analysis of the μg/g brain NE data yielded significant treatment, age, and treatment by age effects (Table 17). Table 18 shows individual comparisons of mean μg/g brain NE levels for the eight treatment groups. These data show that NE levels for T1, T2, T3 and T5 Ss were significantly higher than those of T4, T8, T6 and T7 groups. In addition, NE levels of T1 and T5 Ss were significantly higher than those of T2 and T3 Ss. Comparison of the mean μg/g brain NE levels for the four age levels showed NE to be significantly higher at A3 than at A1, A2, or A4 (Table 19). Comparison of this data for the T8 groups as a function of age showed that NE levels at A3 were significantly higher than at A4 and A1, while they did not differ from the A2 level (Table 20). Mean μg/g brain NE levels for the eight treatment groups are shown in Figure 39. These data for the four age levels are presented in Figure 40. Figure 41 (a and b) illustrates the treatment by age relationship.

Analysis of phenylalanine in milligrams percent plasma yielded significant treatment, age and treatment by age effects (Table 21). Individual comparisons of mg/percent plasma phenylalanine levels for the treatment groups are presented in Table 22. These data show that T6, T7, T1 and T2 groups had significantly higher plasma phenylalanine levels than T5, T4, T8 and T3 groups. In addition, T6 and T7 groups had significantly higher plasma phenylalanine levels than T1 and T2 groups. Individual comparisons of mean mg/percent plasma phenylalanine levels for T8 Ss did not show a significant change in these levels as a function of age (p greater than .05). Mean mg/percent plasma phenylalanine

TABLE 17

ANALYSIS OF VARIANCE:

BRAIN NOREPINEPHRINE (μg/g)

Source	df	MS	F Ratio
Treatment (T)	7	0.05319	7.74*
Age (A)	3	0.53782	98.57*
T X A	21	0.02947	5.40*
Subjects	170	0.00545	

* p less than .01

TABLE 18

TUKEY (a) TEST OF TREATMENT MEANS

BRAIN NOREPINEPHRINE (μg/g)

Treatment	T7	T8	T6	T4	T2	T3	T1	T5
T7 (PCL-LC)					*	*	*	*
T8 (Nor Cont)							*	*
T6 (PCL-HC)							*	*
T4 (Nor Cond)							*	*
T2 (PCL-LE)								
T3 (I^{131})								
T1 (PCL-HE)								
T5 (Pseudo)								

* p less than .01

TABLE 19

TUKEY (a) TEST OF AGE MEANS

BRAIN NOREPINEPHRINE (μg/g)

Age	A4	A1	A2	A3
A4 (28 days)				*
A1 (7 days)				*
A2 (14 days)				*
A3 (21 days)				

* p less than .01

TABLE 20

TUKEY (a) TEST OF AGE MEANS

T8 BRAIN NOREPINEPHRINE (μg/g)

Age	A4	A1	A2	A3
A4 (28 days)				*
A1 (7 days)				*
A2 (14 days)				
A3 (21 days)				

* p less than .01

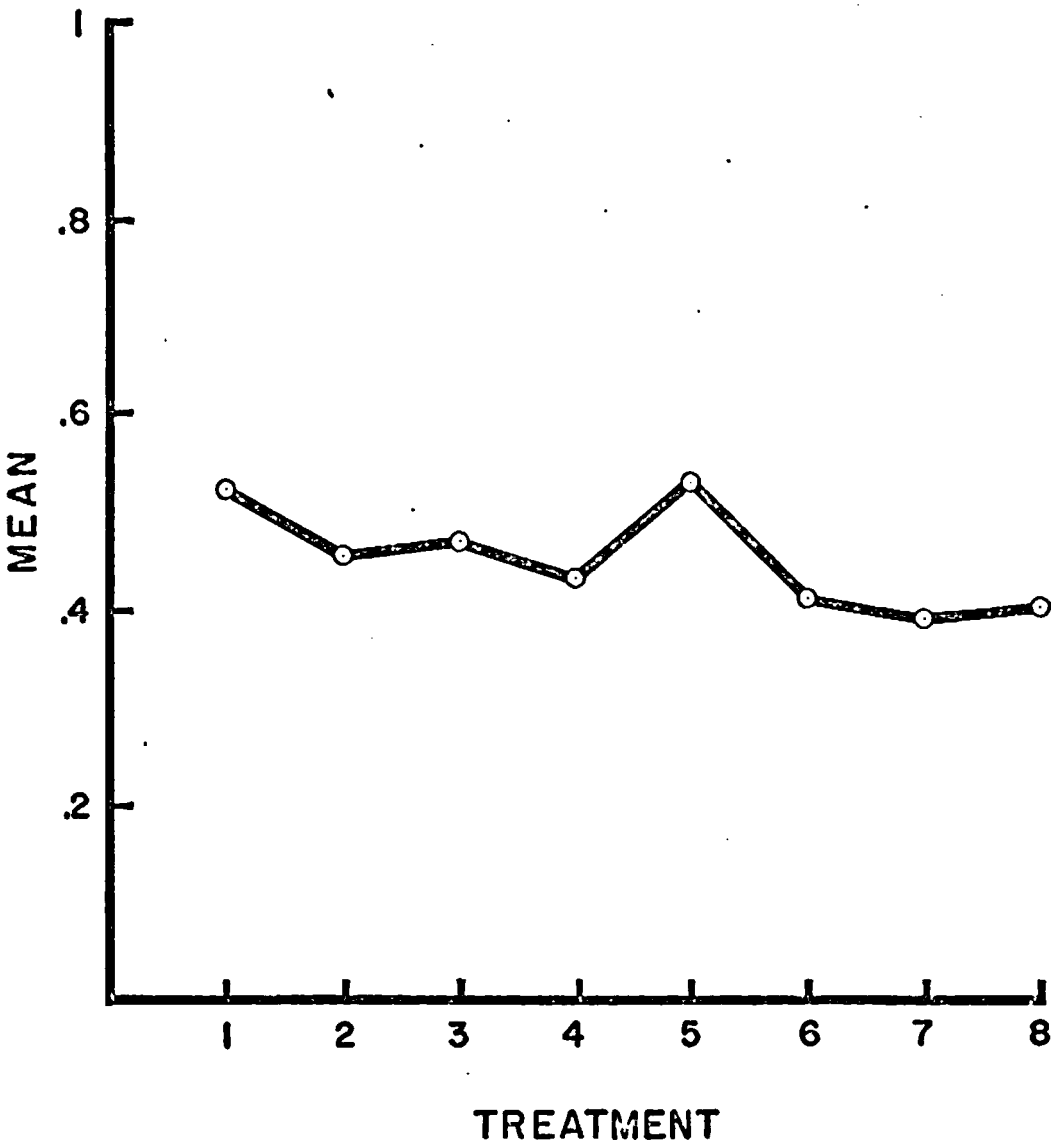


FIGURE 39

MEAN μg/g BRAIN NOREPINEPHRINE

FOR EIGHT TREATMENT GROUPS

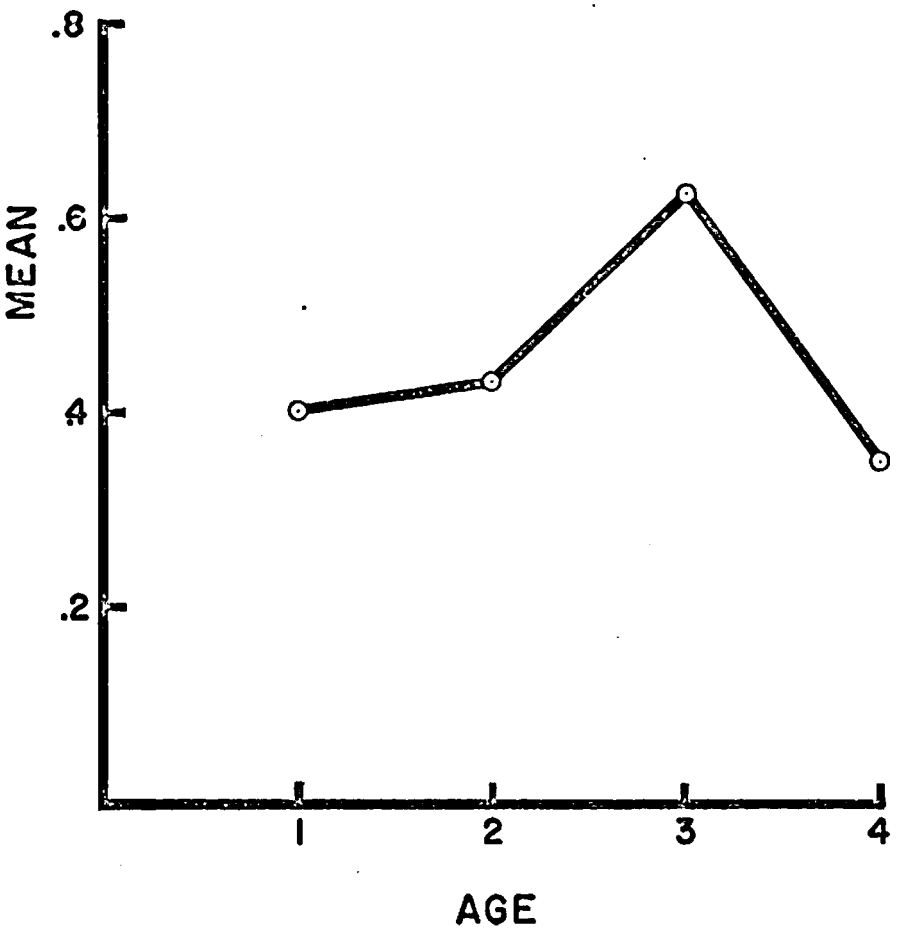


FIGURE 40

MEAN µg/g BRAIN NOREPINEPHRINE

FOR FOUR AGE LEVELS

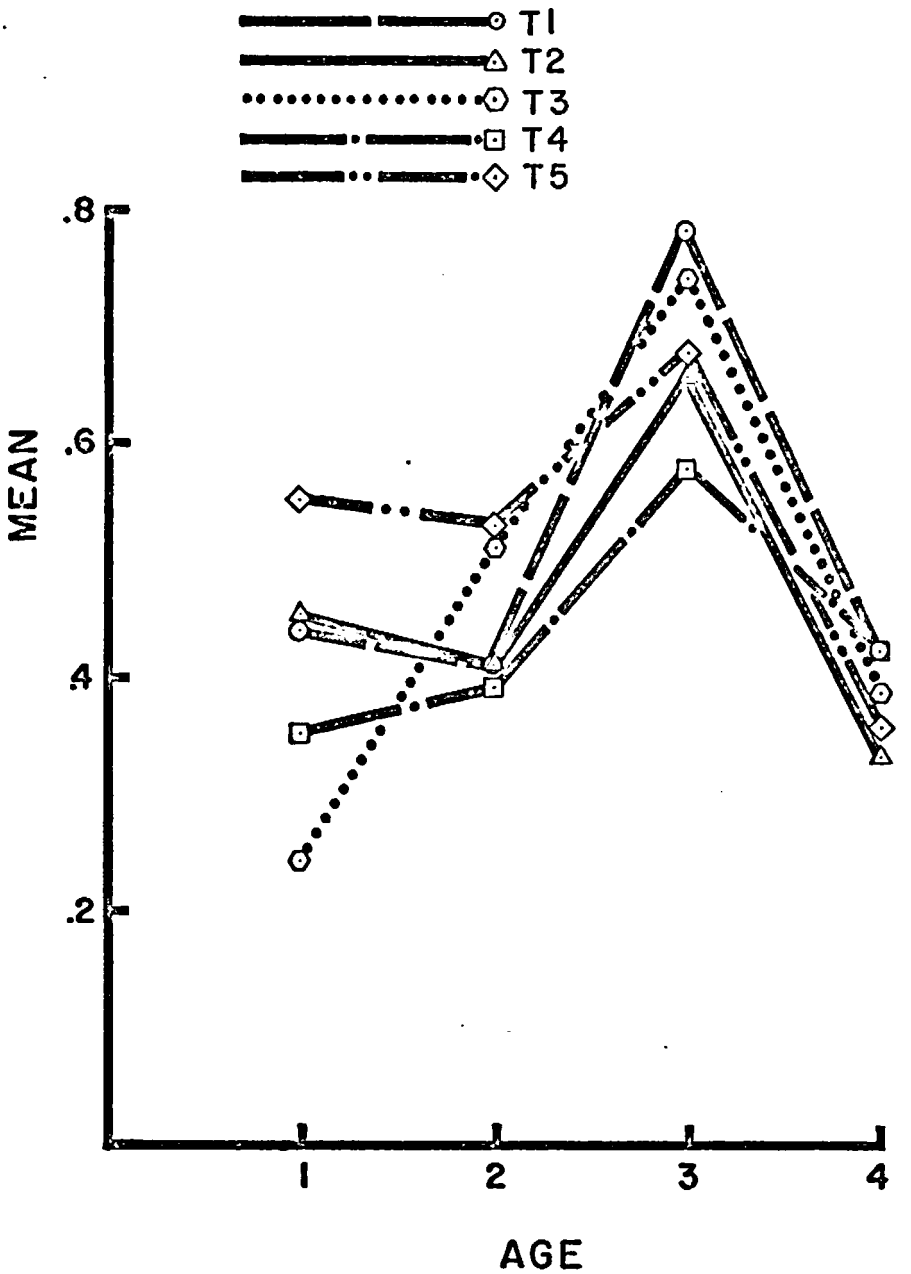


FIGURE 41a

MEAN μg/g BRAIN NOREPINEPHRINE

FOR FIVE CONDITIONING GROUPS AT FOUR AGE LEVELS

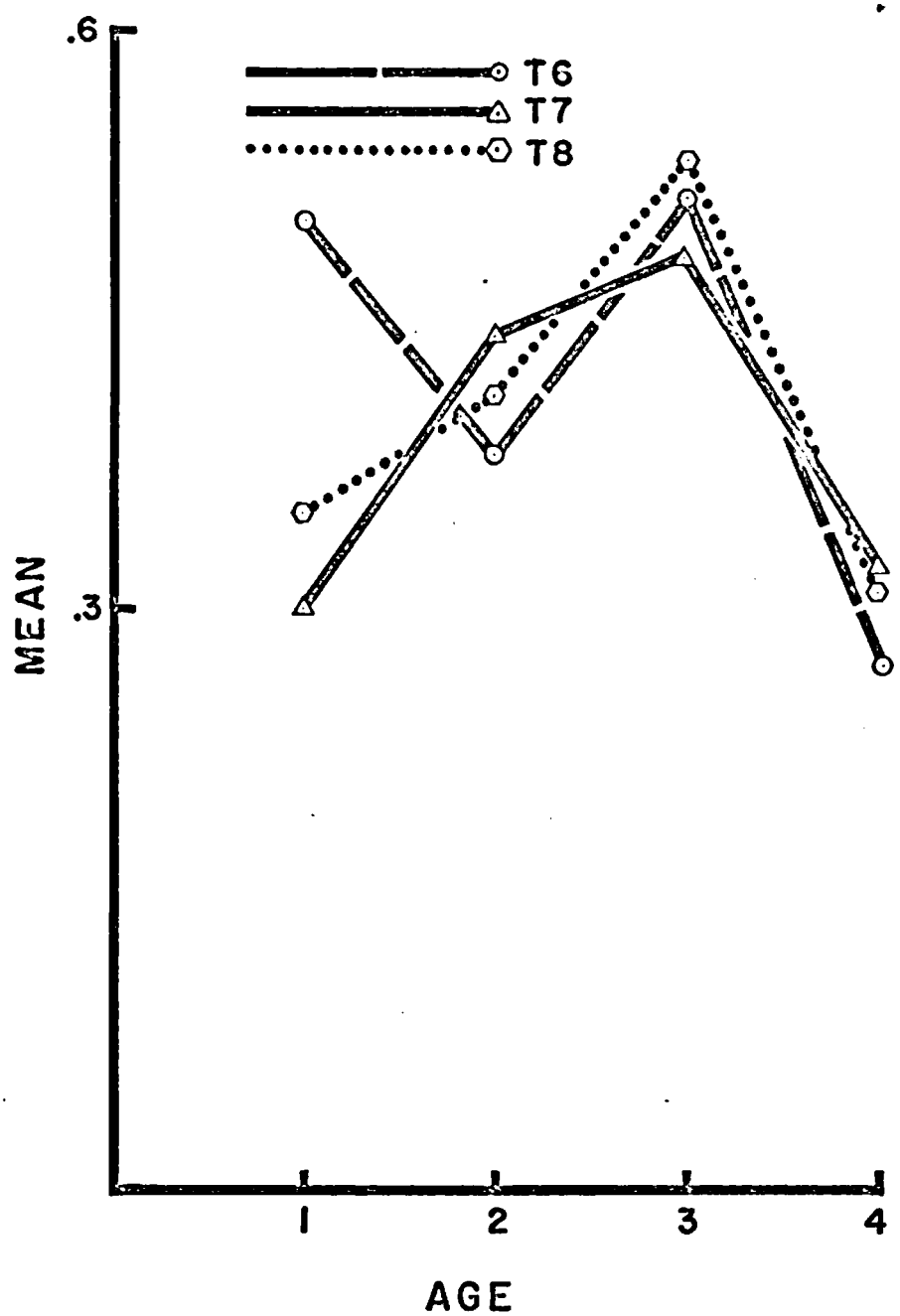


FIGURE 41b

MEAN μg/g BRAIN NOREPINEPHRINE FOR
TWO DRUG CONTROL GROUPS AND
A NORMAL CONTROL GROUP AT FOUR AGE LEVELS

TABLE 21

ANALYSIS OF VARIANCE:

PLASMA PHENYLALANINE (mg/%)

Source	df	MS	F Ratio
Treatment (T)	7	510.84	138.11*
Age (A)	3	170.86	46.19*
T X A	21	57.15	15.45*
Subjects	105	3.69	

* p less than .01

TABLE 22

TUKEY (a) TEST OF TREATMENT MEANS

PLASMA PHENYLALANINE (mg/%)

Treatment	T5	T8	T4	T3	T2	T1	T7	T6
T5 (Pseudo)					*	*	*	*
T8 (nor Cont)					*	*	*	*
T4 (nor Cond)					*	*	*	*
T3 (I^{131})					*	*	*	*
T2 (PCL-LE)							*	*
T1 (PCL-HE)							*	*
T6 (PCL-HC)								
T7 (PCL-LC)								

* p less than .01

levels for the eight treatment groups are shown in Figure 42. These data for the four age levels are shown in Figure 43. The treatment and age relationship is displayed in Figure 44 (a and b). These data show that dl PCL treatment effectively increased plasma phenylalanine levels. Thus, they support the hypothesis that dl PCL treatment would produce the biochemical indices of PKU.

Comparison of weight data yielded significant treatment, age and treatment by age effects (Table 23). The mean weight for the five treatment groups is shown is Figure 45. These data for the four age levels are shown in Figure 46. The treatment by age data are presented in Figure 47. These data show that dl PCL and I^{131} treated groups were strikingly smaller than normal groups. Weight for all groups, of course, increased with age, while the magnitude of the differences between drug treated and I^{131} groups and normal groups also increased with age.

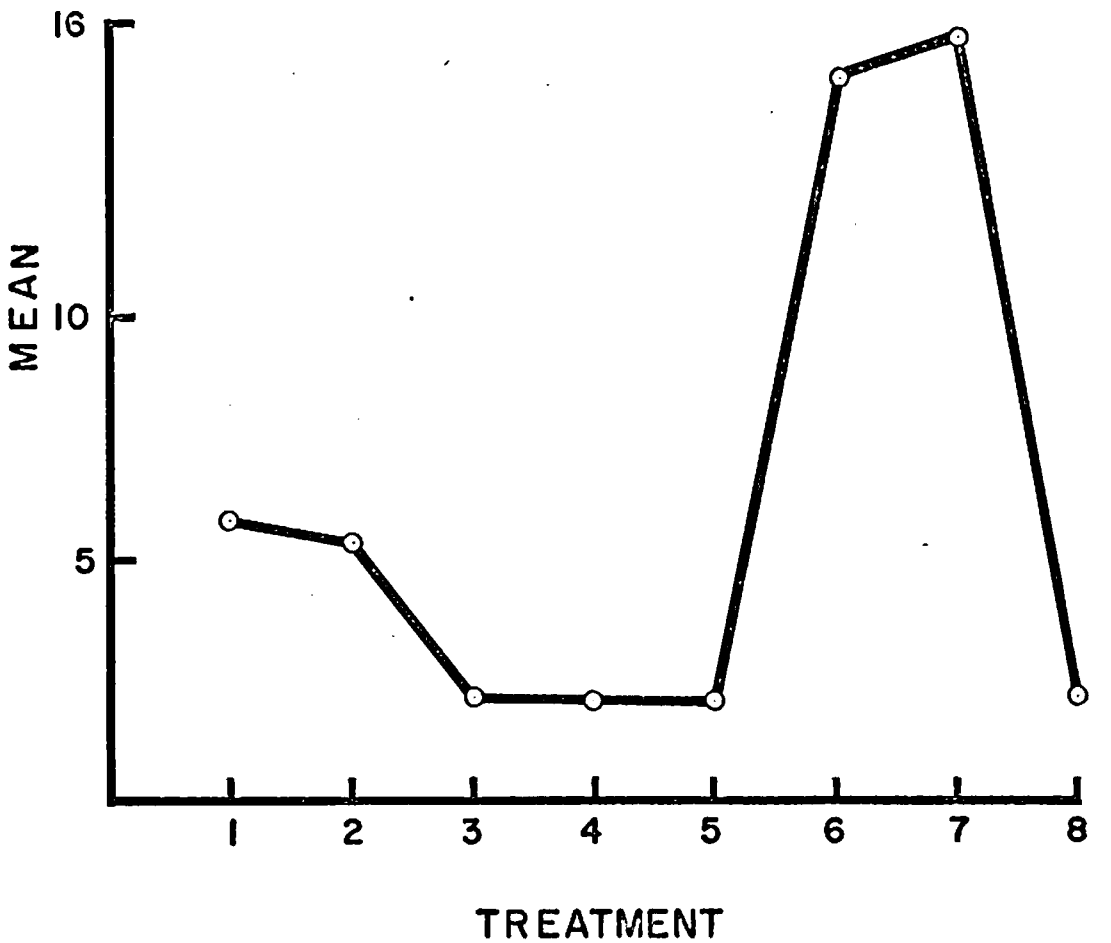


FIGURE 42

MEAN mg/% PLASMA PHENYLALANINE

FOR EIGHT TREATMENT GROUPS

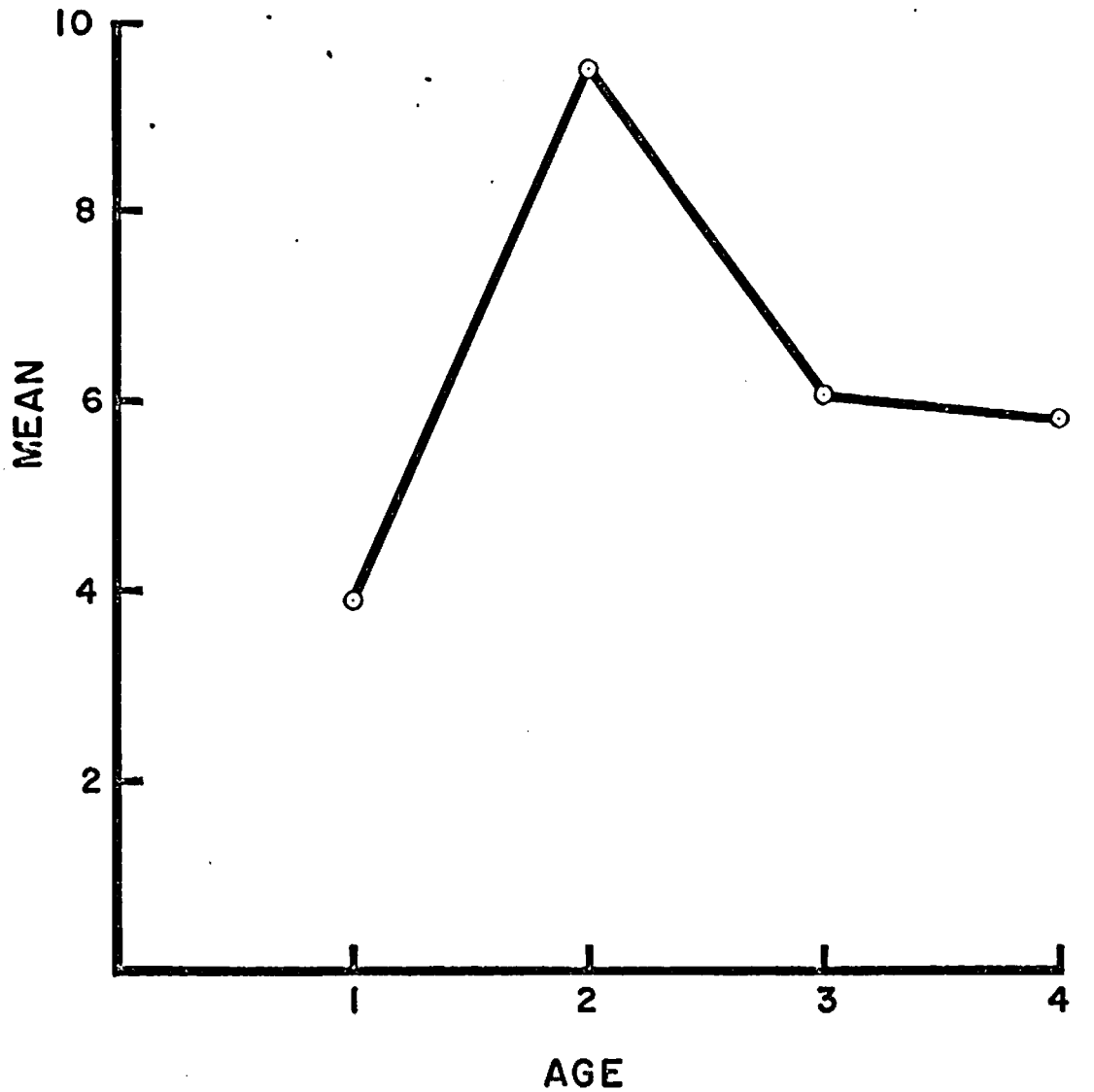


FIGURE 43

MEAN mg/% PLASMA PHENYLALANINE

FOR FOUR AGE LEVELS

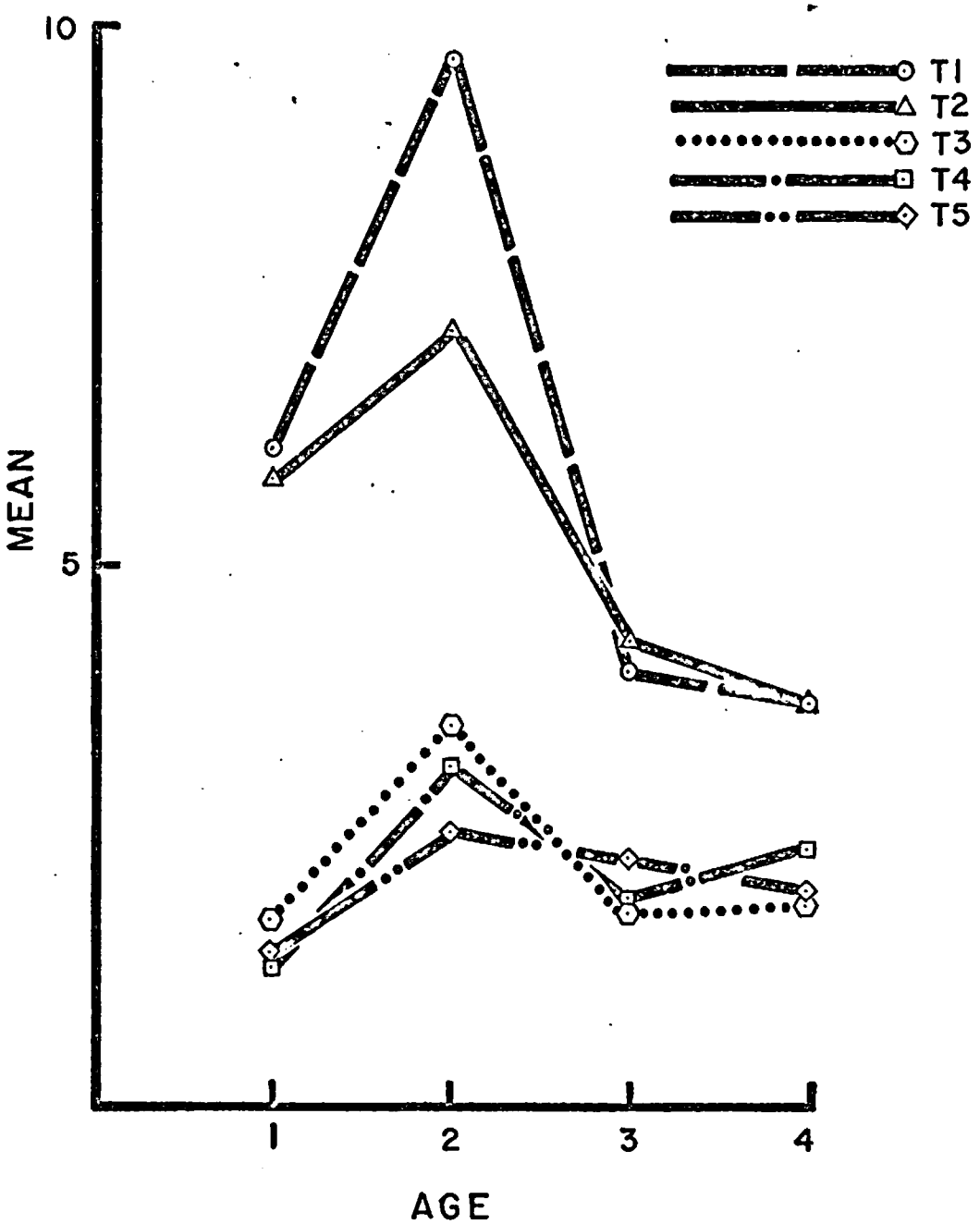


FIGURE 44a

MEAN mg/% PLASMA PHENYLALANINE

FOR FIVE CONDITIONING GROUPS AT FOUR AGE LEVELS

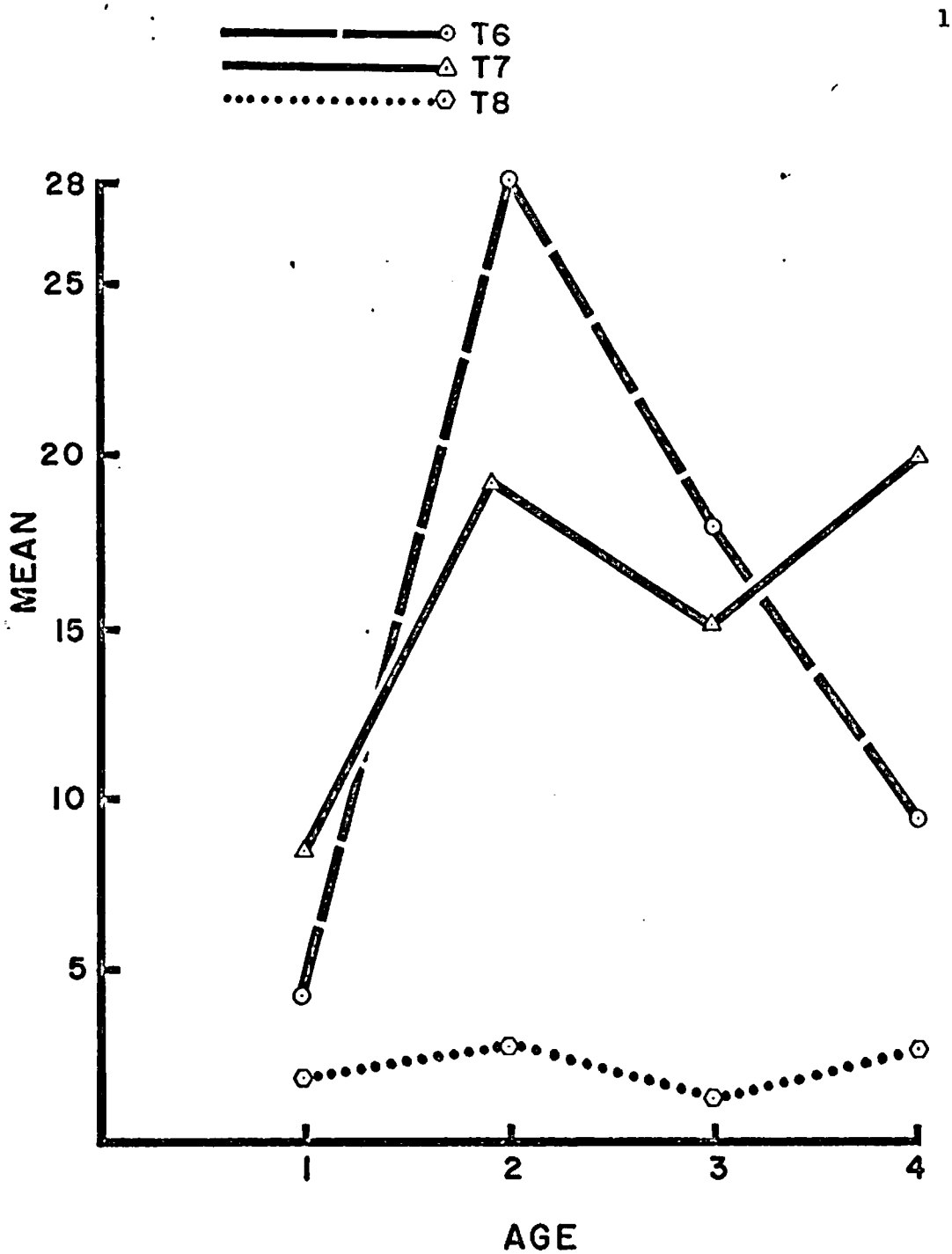


FIGURE 44b

MEAN mg/% PLASMA PHENYLALANINE
FOR TWO DRUG CONTROL GROUPS AND
A NORMAL CONTROL GROUP AT FOUR AGE LEVELS

TABLE 23

ANALYSIS OF VARIANCE:

GRAMS WEIGHT

Source	df	MS	F Ratio
Treatment (T)	4	1395.673	133.68*
Age (A)	3	10525.252	1008.18*
T X A	12	483.329	46.29*
Subjects	180	10.439	

* p less than .01

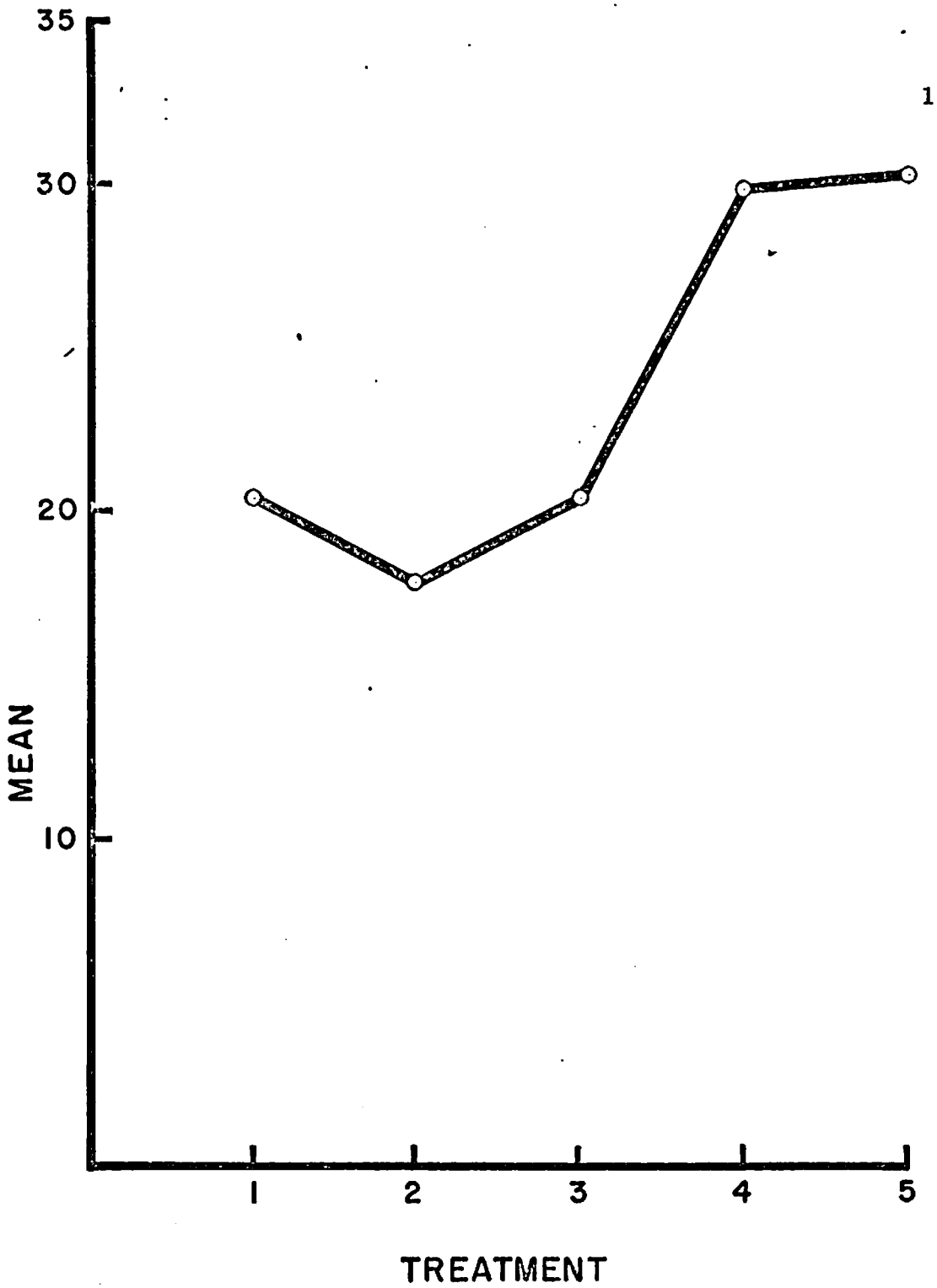


FIGURE 45

MEAN GRAMS WEIGHT FOR FIVE TREATMENT GROUPS

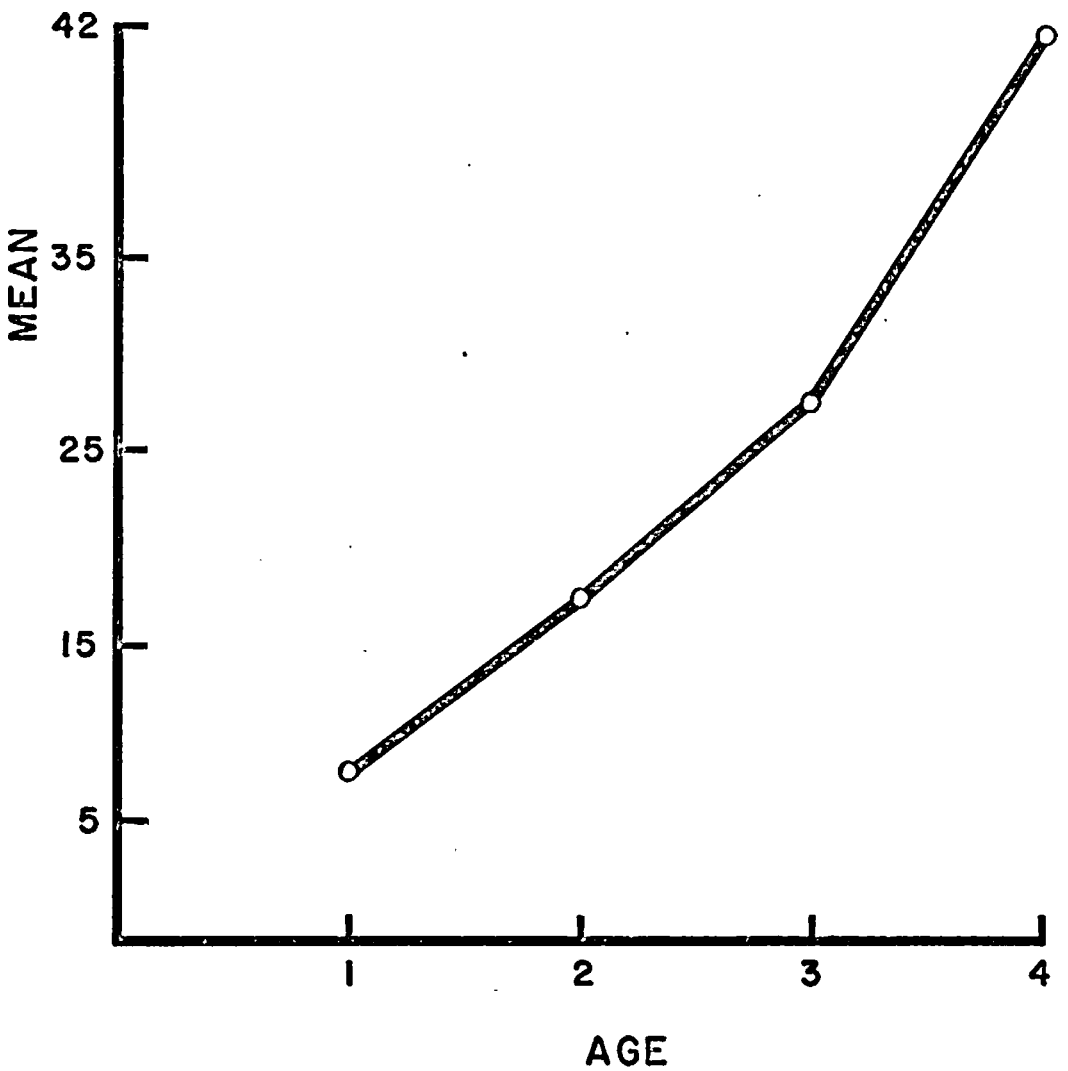


FIGURE 46

MEAN GRAMS WEIGHT FOR FOUR AGE LEVELS

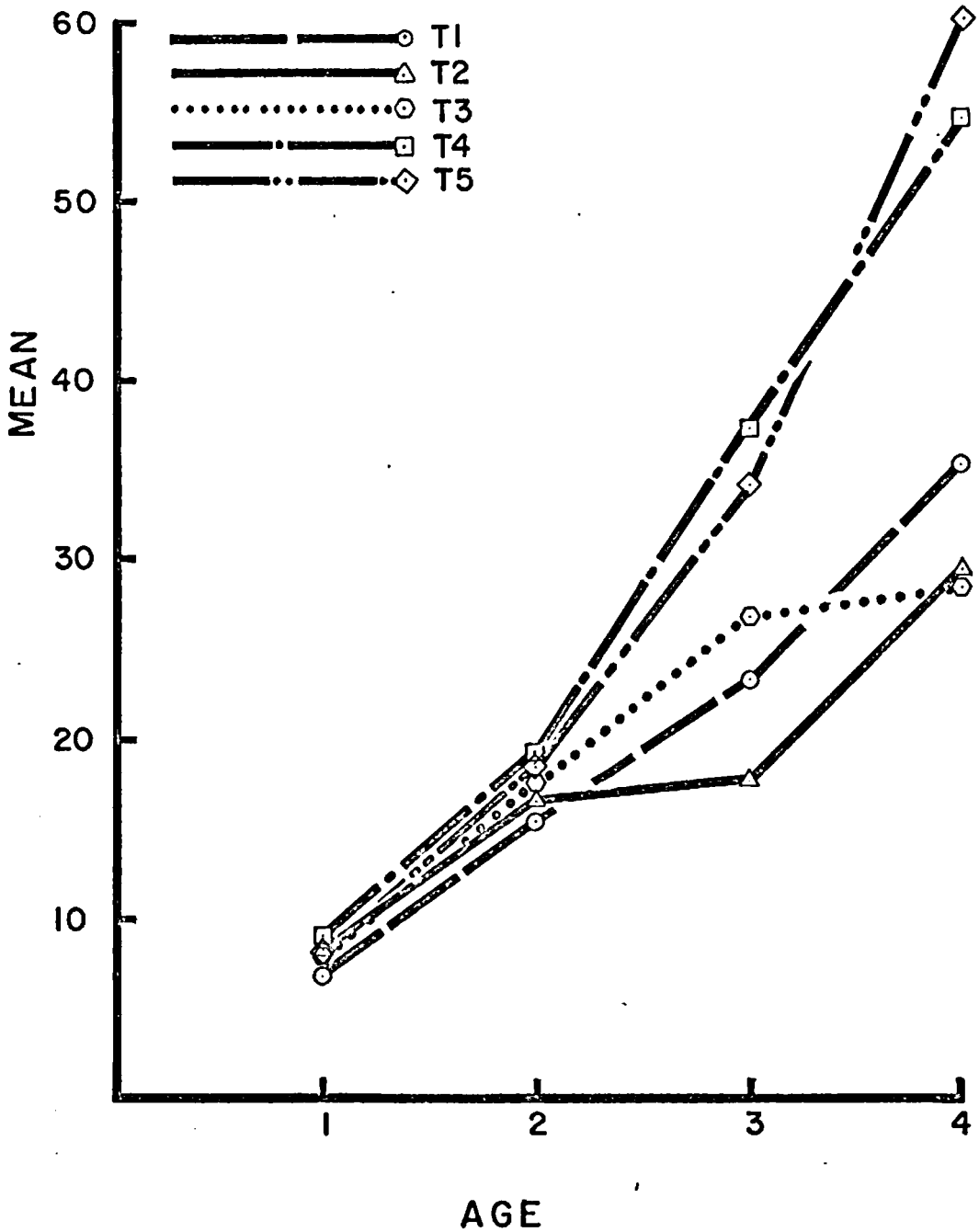


FIGURE 47

MEAN GRAMS WEIGHT FOR FIVE TREATMENT GROUPS

FOR FOUR AGE LEVELS

CHAPTER V

DISCUSSION

Two indices of learning, habituation and the classical conditioned leg flexion response, were utilized to test the hypothesis that learning is, in part, a function of cortical maturation. Normal rats were tested at four intervals during the cortical developmental period to determine if facilitory effects on learning were demonstrable as a result of maturation. Animals receiving treatments designed to simulate cretinism and PKU, mental retardation syndromes which, in humans, are associated with an immaturity of the cortex, were tested also to determine if these treatments resulted in a learning deficit.

The habituation data failed to meet a criterion for homogeneity of variance and, thus, could not be analyzed for both treatment and age effects. Since differences between the treatment groups on the habituation measure would have allowed comparison of results for two learning tasks, the data were analyzed for this factor. As no differences were found to exist between the groups, these data failed to support the report of faster habituation in hypothyroid rats (Eayrs & Lishman, 1955; Eayrs, 1961a). In those investigations, however, the habituation measure was inferred from the degree to which physical alteration of a runway interferred with a previously acquired response (Eayrs & Lishman, 1955) and from the rate of adjustment to a novel environment (Eayrs, 1961a). These would appear to be more complex behavioral tasks than that required of the Ss in this investigation. Habituation measures, similar to that reported in this study, in which the S manifests a failure to respond to

an inconsequential, repetitive stimulus can be considered a primitive form of learning (Thorpe, 1963). At this level, the dl PCL and I^{131} groups did not evidence a learning deficit.

The classical conditioning measures (ACR and TCR) presented strong evidence for the hypothesis of improvement in learning processes with increasing cortical maturation for normal Ss. Within this group, the 21 and 28 day Ss showed better conditioning on TCR and ACR measures than the seven and 14 day groups, while normal Ss, at all ages, performed significantly better than pseudoconditioning Ss.

The ACR levels observed in seven and 14 day normal Ss were 28.2 and 51.7 percent. Gray, et al (1967) report a graph for this measure which indicates levels of 20 percent for six and 28 percent for 12 day Ss. The conditioning levels in the present study, for slightly older Ss, are observed to be higher. In discussing their data, Gray, et al (1967) note that the ACR level they obtained for neonatal Ss, 10 percent, was considerably below the 32 percent reported by Caldwell and Werboff (1962). Since neither report defined the temporal parameters, or the grams pressure, of leg flexion required to constitute a CR, resolution of these discrepancies in absolute levels of conditioning is not possible. There is, however, some indication that the Gray, et al (1957) Ss were less reactive, in general, than those reported by Caldwell and Werboff (1962) and those of the present study insofar as these authors report that during habituation no leg movements were observed in response to the CS.

It is not possible to meaningfully compare the performance of young rats on these measures with that of adult rats as there seem to be no studies reporting successful conditioning of this response in the adult.

However, it is possible to speculate as one recent study presented a graph indicating that ACRs, for a tail movement response which might be considered similar to leg flexion, reached 70 percent (based on 100 trials) in the adult rat (Chacto & Lubow, 1967). This is considerably higher than the 54 percent (based on 80 trials) maximal level found in the current study. Thus the expectation of increasing conditionability in older Ss than those presently reported seems reasonable and clearly needs investigation.

The Caldwell and Werboff (1962) and Gray, et al (1967) studies investigated conditioning as a function of ISI postulating that the optimal ISI becomes shorter with maturation. This question also needs to be empirically resolved both for mature Ss and for Ss of the older ages utilized in the present study.

Brain amine levels have been considered as one index of cortical maturation. In the rat, the neonatal brain levels of 5-HT are low in comparison with those of the adult and rise linearly as a function of age, approximating those of the adult by day 35 (Agrawal, Glisson & Himwich, 1966). The linear increase in brain 5-HT in normal control Ss reported thus, corroborates other investigations of this question (cf. Agrawal, et al, 1966; Nachmias, 1960). In addition, the absolute levels of 5-HT, as a function of age, are very close to those generally reported (cf. Bennett & Giarman, 1965).

Brain NE levels also generally are reported to rise as a linear function of age (cf. Agrawal, et al, 1966). In the normal control Ss reported in this study, NE levels at 21 days were found to be significantly higher than at seven and 28 days, while they did not differ from

those of 14 day Ss. The absolute levels observed in 28 day normal control Ss are somewhat higher than those reported for 25 day Ss, while those of other ages are considerably higher (Agrawal, et al, 1966). In pilot work completed prior to the present investigation, higher brain NE levels than those generally reported were a consistent finding. This finding probably does not represent a measurement error as NE levels for adult Ss, administered dl PCL at early ages but neither drugged nor handled for 11 weeks prior to sacrifice, were found to be normal using the same procedures (Kilbey, et al, 1969). The discrepancy is not seemingly related to strain as the pilot work involved several strains. Stress would seem to be a factor except that NE levels generally are reported to fall as a result of stress (Maynert & Levi, 1964; Barchas & Freedman, 1963; Bliss, Wilson & Zwanziger, 1966). There is, of course, the possibility that these mechanisms are different in the young rat. An investigation of the effects of the various factors involved in the administration of drugs to young animals on brain NE levels is needed to evaluate this data.

The SLF data obtained as a measure of activity prior to and during conditioning indicated that activity levels, in normal Ss, dropped rather sharply from seven to 14 days and remained fairly stable thereafter. The major contribution to this decrease in activity was the disappearance of a seemingly involuntary low-amplitude leg movement which could best be described as a "twitch" response. The data for the measurements obtained to establish the CS and UCS levels showed that skin resistance levels as well as sensitivity to peripheral stimulation decreased, while strength, of course, increased as a function of age.

The range of skin resistance levels reported (5.22 to 67.3 kilohms) is considerably lower than that reported for adult rats (250 to 1,750 kilohms) (Walker & Walker, 1964). However, the decreasing skin resistance as a function of age has its counterpart in the report that human neonates of 15 days of age and over were observed to have much lower skin resistance levels than those of 11 days and under (Richter, 1930).

The report of a developmental pattern of decreasing sensitivity to stimulation indicates that use of invariant stimulus values throughout the cortical developmental period could be expected to give rise to different behavioral consequences at the various ages. These data, thus, support the need for establishing stimulus values within this period in terms of an overt behavioral criterion if the changes in performance are to be interpreted as reflecting changes in learning processes with increasing age.

Both TCR and ACR measures support the hypothesis that I^{131} groups manifested an impaired performance indicative of an intellectual deficit. At none of the ages investigated was the performance of this group comparable to normal Ss of the same age. However, at 28 days this group demonstrated a TCR level considerably above those obtained at earlier ages and comparable to that of the seven and 14 day normal groups. This suggests that with increasing age these Ss achieved some ability to acquire a classical conditioned response. These data corroborate the observations (cf. Eayrs, 1961a) of intellectual deficits in hypothyroid Ss and extend them insofar as they indicate that the learning deficit is detectable in much younger Ss than those previously investigated on a task not previously utilized.

The absence of viable thyroid tissue is generally considered a sufficient criterion for establishment of a cretin condition in the rat (Eayrs, 1961a). In addition, I^{131} treated Ss are reported to be strikingly smaller than normal Ss (Goldberg & Chaikoff, 1949; Eayrs, 1961b). The data reported for the present study show that both these indices were manifested in the I^{131} groups and establish that these groups constituted a cretin analog. The investigation of brain 5-HT and serum phenylalanine levels during the cortical developmental period in I^{131} treated Ss indicated that their metabolism of phenylalanine and 5-HT is normal in all respects. The demonstrated learning deficit is presumed to reflect neuroanatomical and neurophysiological abnormalities known to exist in similarly treated Ss (Eayrs, 1959, 1960) and a possible protein anabolism abnormality (Campbell & Eayrs, 1965).

Eayrs and Lishman (1955) have reported that peripheral sensitivity to electrical stimulation in hypothyroid rats five to 15 days old does not differ from that of normal Ss. The finding that I^{131} Ss did not differ from normals in terms of the current necessary to elicit a minimal leg flexion complimented this report of intact peripheral sensitivity and extended it to slightly older Ss. Pain sensitivity, as measured by the current necessary to elicit a maximum leg flexion, for I^{131} treated Ss appeared comparable to normal Ss at seven and 14 days, while at 21 and 28 days these Ss seemed to be more sensitive than other groups. This finding would not have been predicted from previous work which indicated that the level of shock UCS necessary to optimize active avoidance conditioning in hypothyroid Ss was higher than in normal Ss (Eayrs & Levine, 1963), nor is it congruent with the clinical description

of the nonreactive cretin (Means, 1948). Evidence conflicting with clinical observations of cretins (Means, 1948) was obtained also from the finding of activity levels above those of normal Ss at 21 and 28 days. This result, however, represents the persistence of infantile "twitching" movements in I^{131} treated Ss beyond the age at which they were observed to cease in normal Ss and is congruent with the general picture of immaturity in these Ss.

Skin resistance levels in cretin Ss were observed to be higher than those of normal Ss and were observed to increase as a function of age while those of other groups decreased. These data may indicate derma and/or sweat gland abnormalities in this group and may relate to the clinical observation of dry skin often reported for hypothyroidic humans (Means, 1948).

Performance deficits congruent with the clinical description of impaired intellectual functioning in human phenylketonurics (Nyhan, 1960) were demonstrated in dl PCL treated groups which manifested significantly lower TCR and ACR levels at all ages tested. There were very few indications of any learning in these Ss, although there was a marginal increase in TCR measures as a function of age and ACRs were found to increase slightly over blocks. On both measures, the high dosage dl PCL groups performed worse than the pseudoconditioning groups, while the low dosage dl PCL groups performed somewhat better.

The demonstration of significantly lowered brain 5-HT levels in dl PCL treated groups as well as the finding of significantly increased plasma phenylalanine levels supported the suggestion that PCL would be useful in establishing the biochemical indices of PKU (Koe & Weissman,

1968) and demonstrated that these indices can be established within the cortical developmental period.

The serum phenylalanine levels for the drug control groups are, in some cases, below the 20 mg/percent level suggested for establishing an experimental PKU analog (Karrer and Cahilly, 1965). However, the range of serum phenylalanine levels in PKU patients is large. Nadeau and Fortin (1965) report levels between 10.6 and 20.6 mg/percent in a group of untreated PKU patients, and in another group the range is reported to be between 15 and 72 mg/percent (Partington & Lewis, 1963). Since there is no correlation between the serum phenylalanine level and the intellectual deficit (Crome & Pare, 1960), it is difficult to establish a lower bound for this measure indicative of the syndrome, and current clinical practice considers levels above 6 mg/percent plasma phenylalanine "presumptive positive" for PKU (Guthrie, cited in Sigma Chemical Company, 1966). The mean plasma phenylalanine levels for seven day Ss are not comparable to PKU patient levels, nor are those of the T6 group at 28 days, but all other means are well within the range reported. The finding of an initial increase followed by a reduction in serum phenylalanine levels as a function of age is congruent with the developmental pattern established in human phenylketonurics (Partington & Lewis, 1963).

After seven days of age, the normal increase in 5-HT levels as a function of age was observed in the dl PCL conditioned groups. However, the mean 5-HT level for conditioned dl PCL treated Ss was 38 percent that of normal Ss which is roughly comparable to the 46 percent normal serum 5-HT levels reported for PKU patients (Pare, Sandler & Stacey, 1957). While as employed in this study dl PCL treatment resulted in somewhat lower

levels of both plasma phenylalanine and brain 5 HT than those reported for PKU patients, the dl PCL treated Ss may be considered to constitute a PKU analog on the basis of the biochemical measures.

There is, however, one biochemical index of PKU which needs investigation in relation to the use of dl PCL to establish an animal analog of the condition. Partington and Lewis (1963) in a study of untreated, fasting PKU patients found that plasma tyrosine levels for patients younger than 11 months are significantly higher than normal while those of patients over 11 months of age are significantly lower than normal. Decreased tyrosine levels have been established following acute administration of PCL to the adult rat (Lipton, et al, 1967). However, the relationship between administration of PCL and serum tyrosine levels as a function of age is not known and needs investigation to provide a complete assessment of the appropriateness of this drug for establishing the biochemical indices of PKU.

The report of significantly lower weight of dl PCL treated Ss is consonant with the finding that young PKU patients are often under age standards for height and weight and that adult PKU patients tend to be smaller than average (Knox, 1960). The degree to which this finding of smaller size characterizes the PKU syndrome is not known, however, as the finding could be attributed to problems inherent in feeding the mentally retarded (Knox, 1960). The question is open to investigation as at least one author reports normal physical development for PKU patients (Nyhand, 1963). Until there is additional anthropometric data available for PKU patients, it will be difficult to judge the appropriateness of the low body weights in dl PCL treated animals.

The finding that dl PCL treated Ss did not differ from normals in terms of peripheral sensitivity, while they did on a measure of pain sensitivity has its counterpart in the observations of Tenen (1967) who reported a similar finding for the adult, PCL acutely treated rat. As this phenomenon has been reported by Harvey and Lints (1965) who used medial forebrain bundle lesions to reduce 5-HT levels rather than drug administration, it seems to be a function of 5-HT levels independent of changes in phenylalanine levels. Tenen (1967) also has suggested that PCL treated Ss may manifest an increased sensitivity to touch as well as pain. The finding of greater sensitivity to vibro-tactile stimulation in low dosage dl PCL treated Ss would support this suggestion, while the finding of reduced sensitivity in high dosage dl PCL treated Ss at older ages would not.

PKU patients are often found to be hyperactive (Knox, 1960). The activity measures obtained in this study which showed dl PCL treated Ss to be more active than normal Ss is consonant with the behavioral observations. In addition, the activity levels of high dosage drug groups were above those of the low dosage drug groups.

Observations of performance decrements in Ss receiving chronic administration of PCL have been reported (Schlesinger, et al, 1968). The conditioning data of this study extended this finding to animals tested 72 hours after the last drug administration. The observation of an interaction between 5-HT levels and pain sensitivity has also been extended to younger Ss than previously investigated (Harvey & Lints, 1965; Tenen, 1967).

The biochemical and behavioral data of this study support the

hypothesis that dl PCL treatment would establish a PKU analog. It is not known if neonatally dl PCL treated Ss would continue to evidence learning deficits when tested after longer drug free periods and, of course, to develop a truly useful analog a permanent deficit must be demonstrated. However, the report of learning deficits in Ss tested during drug administration (Schlesinger, et al, 1968) and the present report of deficits in Ss tested 72 hours post-drug administration in conjunction with the evidence that neonatal dl PCL treatment significantly lowers the mature brain weight (Kilbey, et al, 1969) suggest that a behavioral deficit may also be found at maturity in animals receiving dl PCL during the developmental period. The rat may yet prove to be an appropriate animal for experimental delineation of the relationship between the biochemical and behavioral indices of PKU.

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APPENDIX A

EXPERIMENTAL PARADIGM

1. Calibrate myograph
2. Position S
3. Obtain basal skin resistance level
4. Measure three maximum amplitude spontaneous leg flexions
5. Determine shock level which elicits a minimal (1 mm.) leg flexion
6. Determine shock level which elicits three, consecutive maximum leg flexions
7. Determine S's responsiveness to twelve levels of vibro-tactile stimulation
8. Complete habituation paradigm
9. Complete conditioning paradigm
10. Mark S and return him to home cage
11. Sacrifice S on morning of the day following conditioning

APPENDIX B

VIBRO-TACTILE THRESHOLD DETERMINATION SCHEDULE

Trial Number	Value*	Trial Number	Value	Trial Number	Value
1	.50	21	2.75	41	2.00
2	.75	22	1.00	42	2.25
3	2.00	23	.25	43	.50
4	1.75	24	2.25	44	3.00
5	.25	25	.75	45	.25
6	1.00	26	1.00	46	1.50
7	1.50	27	2.00	47	2.75
8	1.75	28	1.75	48	3.00
9	2.00	29	2.50	49	1.00
10	2.50	30	2.25	50	2.25
11	.50	31	.25	51	3.00
12	2.25	32	.50	52	1.00
13	2.75	33	1.50	53	1.75
14	1.25	34	.75	54	.75
15	2.75	35	1.25	55	1.25
16	3.00	36	1.25	56	.25
17	2.50	37	.50	57	2.00
18	3.00	38	1.75	58	2.50
19	.75	39	2.50	59	1.50
20	1.25	40	1.50	60	2.75

* Values refer to dial settings on the Heath Audio Generator described in the Apparatus Section.

APPENDIX C

CONDITIONING SCHEDULE

(CS-UCS PAIRED)

Pair Number	Minutes	Interval
0	3'	
1	4'	60"
2	4'40"	40"
3	5'30"	50"
4	6'40"	70"
5	8'	80"
6	8'40"	40"
7	10'	80"
8	11'10"	70"
9	12'	50"
10	13'	60"

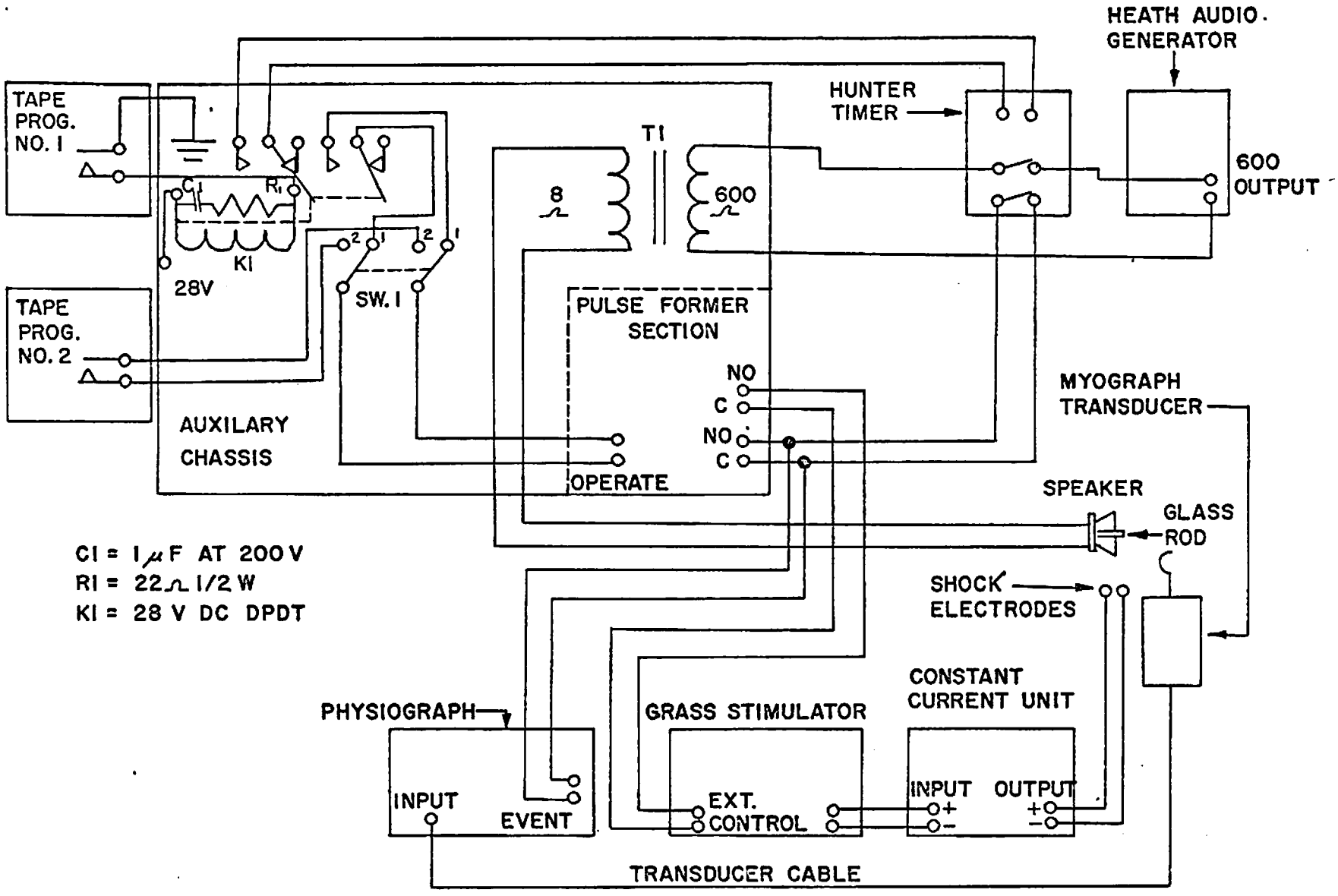
APPENDIX D

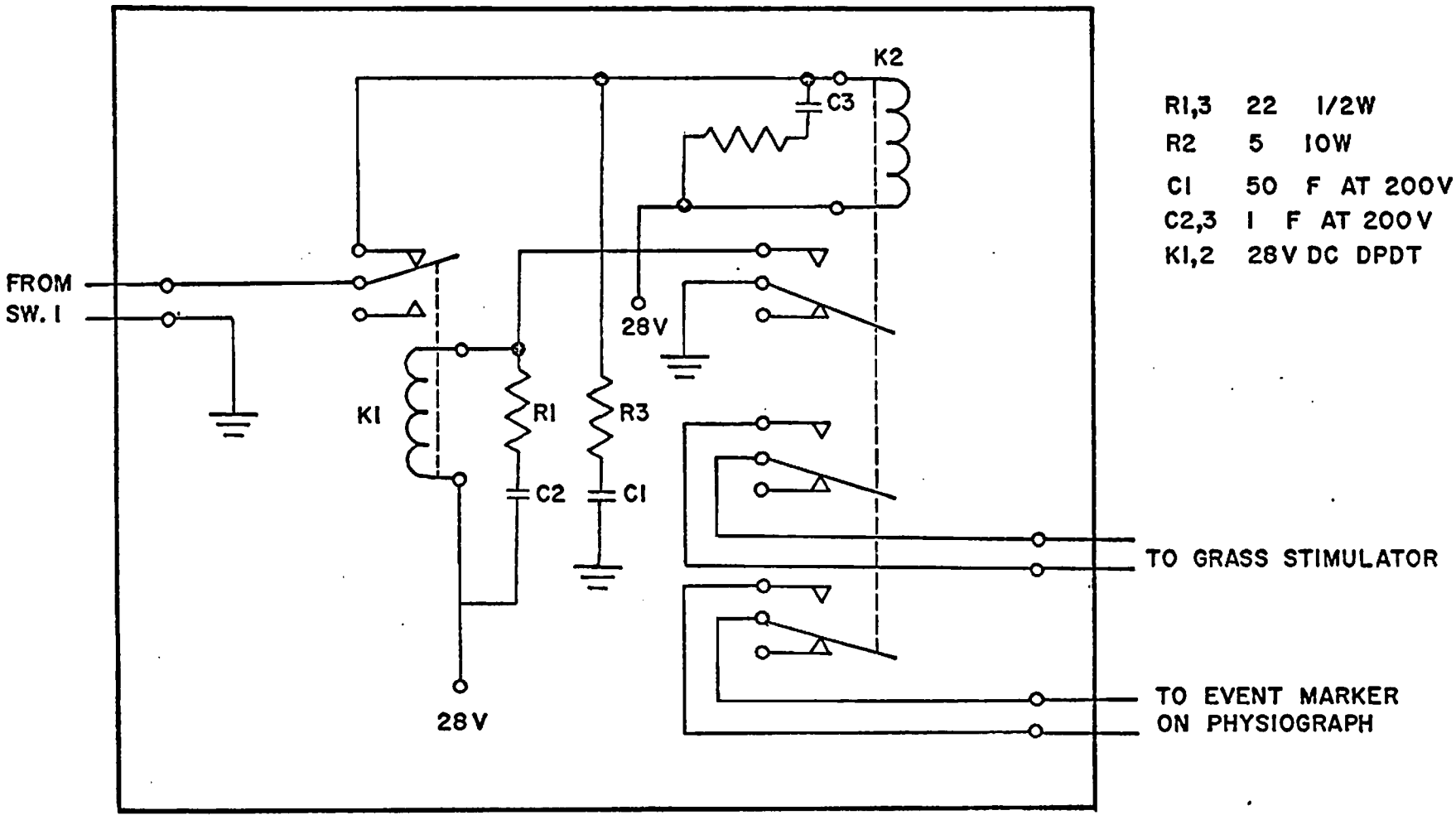
PSEUDOCONDITIONING SCHEDULE

	Stimulus	Interval
0	-	3'
1	CS	27"
2	UCS	20"
3	UCS	40"
4	CS	8"
5	CS	33"
6	UCS	37"
7	CS	27"
8	UCS	22"
9	CS	53"
10	UCS	7"
11	UCS	35"
12	CS	50"
13	CS	12"
14	CS	47"
15	UCS	9"
16	UCS	30"
17	CS	25"
18	CS	65"
19	UCS	5"
20	UCS	38"

APPENDIX E

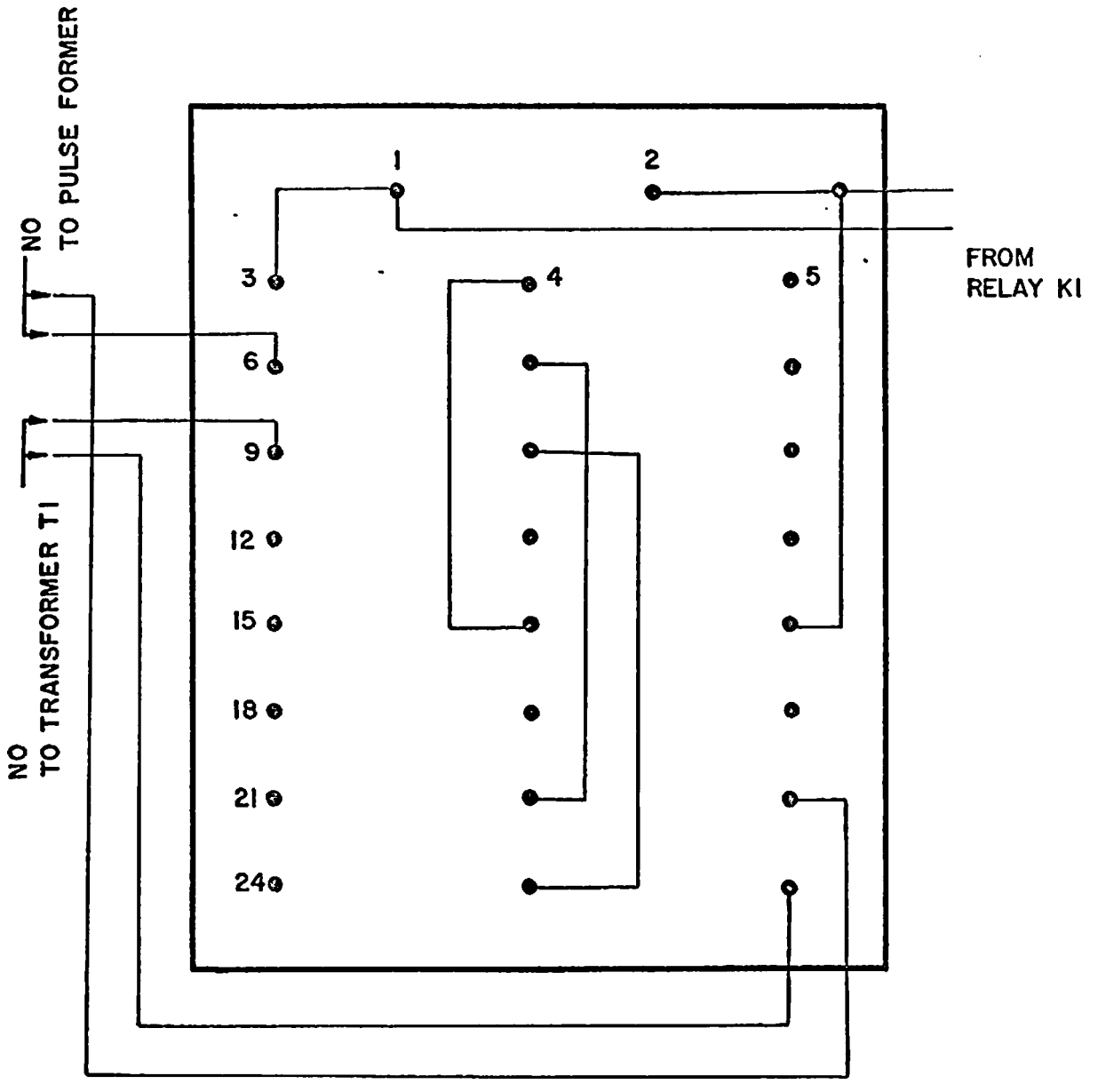
SCHEMATIC DIAGRAM OF THE APPARATUS


HEATH AUDIO GENERATOR
HUNTER TIMER
TAPE PROG. NO. 1
TAPE PROG. NO. 2
T1
8
600
600 OUTPUT
C1
R1
K1
28V
SW. 1
PULSE FORMER SECTION
AUXILARY CHASSIS
NO
C
NO
C
OPERATE
MYOGRAPH TRANSDUCER
SPEAKER
GLASS ROD
SHOCK ELECTRODES
CONSTANT CURRENT UNIT
PHYSIOGRAPH
GRASS STIMULATOR
INPUT
EVENT
EXT. CONTROL
INPUT
OUTPUT
TRANSDUCER CABLE
C1 = 1μF AT 200V
R1 = 22Ω 1/2 W
K1 = 28 V DC DPDT



R1,3	22	1/2W
R2	5	10W
C1	50	F AT 200V
C2,3	1	F AT 200V
K1,2	28V DC DPDT	

PULSE FORMER SECTION



HUNTER TIMER WIRING