SKELETAL GROWTH AND DEVELOPMENT OF THE HUMAN FETUS: EFFECT OF MATERNAL AND NUTRITIONAL FACTORS

by

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B.H.E., University of British Columbia, 1966

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE IN HUMAN NUTRITION

in the Department of
Home Economics

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1971

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A B S T R A C T

Growth is associated with the availability of essential nutrients and it seems possible that these nutrients could affect the growth mechanism involved in skeletal development. To test this hypothesis 76 normal human fetuses aged 9 to 20 weeks were collected from therapeutic abortions. Sex, weight, length, head circumference, foot length and a skeletal index were recorded; developmental age was calculated from crown-rump length, and gestational age estimated from the mother's menstrual history.

Bones from the right arm and leg were removed and cleaned for biochemical analysis. Calcium, inorganic phosphorus, magnesium, sodium and collagen content of 60 femora and humeri were determined, after length, fresh weight, constant dry weight and fat-free weight were recorded. Length of ossification in the bones of the left arm and leg was measured via silver radiography. Assuming bilateral symmetry, biochemical and physical data could then be compared. All fetal data were grouped according to developmental age: 9-10, 11-12, 13-14, 15-16, 17-20 weeks. Analysis of variance and Duncan's New Multiple Range Test were performed to determine the significance of group effect. Simple linear regression was executed on the whole

range of data to detect which variables best predicted other variables.

Maternal information was obtained from an interview and from medical records at Vancouver General Hospital. Age, weight, height, birth weight, parity and gravidity of the mother were recorded. A socio-economic index was calculated. Adequacy of maternal diet during pregnancy was assessed from a daily pattern recall, food frequency and preference questions. These data were used to calculate a total nutrition score and a protein score. Maternal data were coded as potential independent variables and multiple regression analysis performed against fetal dependent variables.

As developmental age of the fetuses increased, the fresh length, dry weight and length of ossification also increased in both humerus and femur, as did the calcium and phosphorus content. In most cases long bone growth as measured by these variables advanced proportionately with fetal age. Thus group means of most variables were significantly different from each other when divided into five 2 week age periods. Water content dropped proportionately with age, reflecting bone mineralization. Sodium content fell markedly in fetal bones after 10 weeks. Magnesium and collagen remained constant. Fat extraction did not change the dry weight of the bones.

Statistical correlation was found between physical and biochemical data. Generally physical variables were best predicted by other physical variables. Biochemical composition of the femur could best be predicted from corresponding data in the humerus. When gestational age was plotted against physical or biochemical variables, statistical correlation was weaker.

The correlation found between fetal variables and maternal age, parity, weight and socio-economic status would indicate a diversity of factors influencing fetal growth. Whereas protein score of maternal diet was not statistically related with fetal parameters, general nutrition score showed a consistent, positive correlation with length and dry weight of the femur and humerus. This relationship was statistically significant when developmental or gestational age remained constant. The results of this study suggest that nutrition of the pregnant woman is positively correlated with some indices of skeletal growth and development of the human fetus.

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Acknowledgment is due to: Dr. Betty Poland, for embryological guidance and for access to both mothers and fetuses; Dr. Poland's staff, for their cooperation and technical assistance; Dr. George Eaton, for statistical advice and negotiatons with the computer; and Dr. Birkbeck along with the Division of Human Nutrition.

REVIEW OF LITERATURE

One of the fundamental features of development is growth, defined as an increase in spatial dimensions and weight. Growth may be accomplished through increases in the number of cells, the size of individual cells, or the amount of intercellular substance (1). Fetal growth of a particular organ or tissue is usually produced by all three components simultaneously. The observation by Schultz in 1926 (2) in his comprehensive treatise on the fetal growth of man and other primates, that more is known about growth in the embryonic and postnatal periods than about the fetal periods, is still valid today.

Until recently the human fetus was considered to have a relatively constant growth rate so that a small baby was necessarily a premature one. Over the last 20 years obstetricians and pediatricians have become aware that the human fetus, like all other living things, grows at a variable rate. It has also become clear that the size a baby has attained relative to the period of gestation is important in determining the hazards it will face in the perinatal period (3-13).

A. Parameters of Fetal Growth and Development

1. Birth Weight

Since it is obviously impossible to study human fetal growth longitudinally, one must rely on birth weight curves to compare fetal growth. Such curves are based on the assumption that birth weights after various lengths of gestation are representative of normal fetal weight at those times. Published charts such as those of Battaglia (14), Lubchenco (15, 16) and Usher (17) are of limited validity so far as normal fetal development is concerned, as information has been obtained from premature births or spontaneous abortions. It is not usually known whether the mishap was due to uterine anomalies, placental defect or fetal abnormality, but at least it is unjustified to accept the level of fetal growth as resulting from normal gestation.

Present workers in the field of human prenatal development continue to show how difficult it is to demonstrate cause and effect in growth. Extensive evidence from human and animal studies indicates that birth weight is primarily determined by factors relating to uterine environment rather than by the genetic constitution of the fetus (18, 19). Determinants of birth weight, varying in significance and directness of their effect, have been considered by various authors (20, 21, 22). These include: racial origin (23, 24), period of gestation(25, 26), type and amount of prenatal care (27, 28), social and economic

status (29, 30, 31), maternal age (32), maternal weight and height (33-38), maternal cigarette smoking (39, 40), maternal disease (41, 42), maternal prenatal nutrition (42-46), maternal education (45), maternal occupation (47),parity and birth order (48, 49), sex of infant (84), geographical location and season (143).

For example, Gruenwald (50) feels that the effi-cency with which the maternal organism satisfies the needs of pregnancy can be judged by fetal growth. New values for birth weight in relation to gestational age have been proposed, and are illustrated in Figure 1 (51). According to Gruenwald it is likely that the normal birth weight curves of various population groups do not differ from one another during the first half of the third trimester or longer. The linear course is indicative of unrestrained growth regulated by the growth potential of the fetus in the presence of an adequate supply line. A time comes when support is no longer adequate for unrestrained growth. The lower the level of growth support received from the mother via the placenta, the earlier the departure from the straight line growth, and the lower is the birth weight at term (see graph). There is a trend toward higher birth weights within most population groups as a result of improved nutritional, socio-economic and medical conditions. The spectacular change in birth weights in Japan during a 20 year period was caused only by better fetal growth and

not by an increase in duration of pregnancy (21). It would be interesting to apply this hypothesis to Meredith's world-wide comparative treatise of birth weights (52).

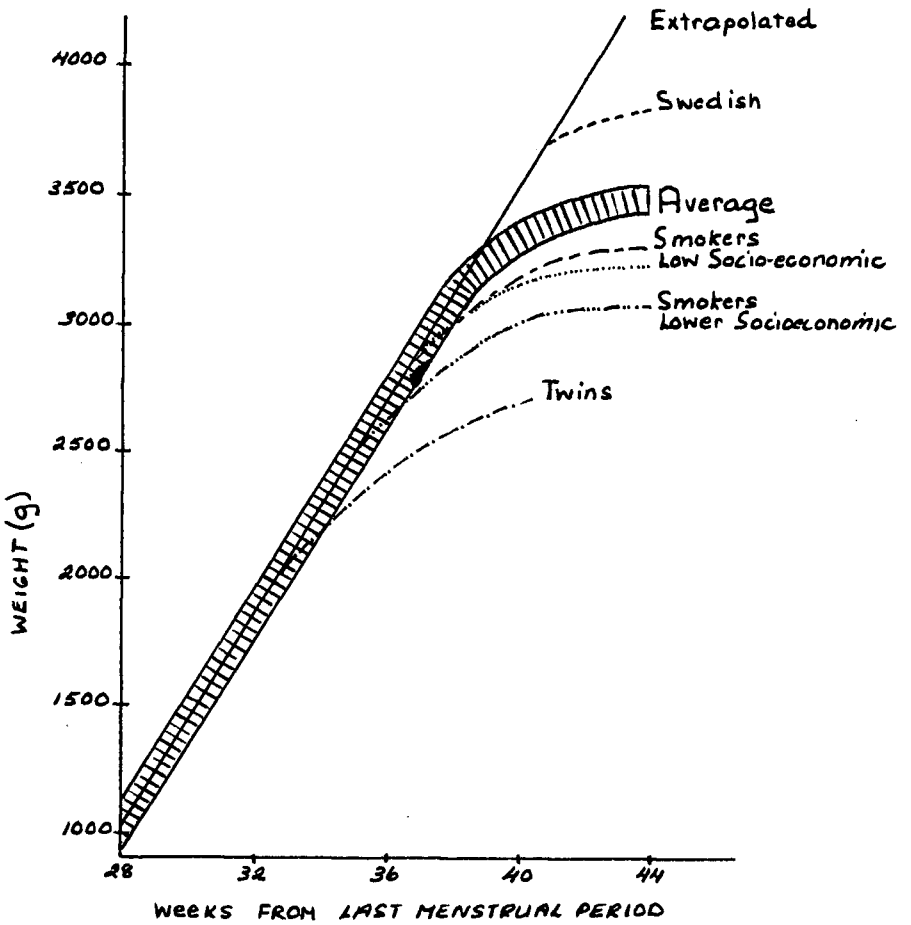


Figure 1. Semidiagrammatic presentation of fetal growth (determined from birth weight) of several population groups (51).

2. Skeletal Growth and Development

a) Ossification and Growth

Histogenesis of human cartilage and bone has been well described (53, 54, 55, 56). The forerunner of the skeleton in the fetal body is formed as a cartilaginous framework, and this begins to calcify at about the eighth week of gestation. Wallgren (57) has made a detailed microradiographical study of the process of ossification of fetal bone and has shown that ossification in the long bones begins at the center of the cartilagenous model. A thin layer of calcified bone matrix is laid down between the perichondrium and that portion of the shaft containing hypertrophic cartilage cells, and by extending around the shaft, forms a ring or collar. This collar is incomplete at first, and the rate of development varies from one type of bone to another and even the long bones do not all develop equally rapidly. The classic review of the histogenesis of cartilage and bone using the fetal humerus as an example has been presented by Streeter (58). Recently Gray has outlined the prenatal development of the human femur (59) and humerus (60).

Many tables are found in the early literature categorizing the developmental sequences of both membranous and endochondral ossification (61-68). Using Streeter's or Boyd's (69) method of staging human fetuses, a rough approximation of age can be obtained by plotting crown-

rump length on a standard curve. Time of occurence of primary ossification centers can then be related to developmental age. Several limitations of this procedure must be considered. Fetuses of a given age could vary considerably in length, and two fetuses of similar length may differ significantly in degree of development. Again, the majority of studies have been performed on spontaneously aborted or still-born fetuses, development of which may not necessarily be considered normal. Initial recognition of an ossification center varies with the technique used, and the following methods are listed by Noback (70) in what he considered to be their order of decreasing sensitivity: sectioning, clearing and alizarin staining, radiography and gross dissection. With the development of heavy metal staining by Hodges (71) in the fetal pig and O'Rahilly (72) in the human fetus, silver radiography is now considered to be as sensitive as alizarin staining.

Regardless of technique, several principles concerning ossification and growth have evolved. Ossification centers may be regarded as indices of anatomical maturity, and variability in their appearance may reflect the variability of maturation of the skeletal system. One part of the body is a criterion of normalcy for the other parts; whereas one of a pair of bilateral ossification centers may appear at a different time than the other center of the pair, the degree of such asymmetry is usually slight before birth. Uses of this principle as a diagnostic tool are many.

Radiological assessment of fetal maturation in utero can predict the date of delivery more accurately than menstrual history (73, 74). Epiphyseal maturation of certain centers at birth is gaining recognition as a means of estimating the age of the infant at birth (75, 76, 77) and of predicting neonatal respiratory distress syndrome (78). Use of radiological techniques to determine bone age in children (79) is well known. It would seem of practical significance to have a similar standard curve of fetal bone ages from eight weeks gestation to term; unfortunately these data cannot be found in the literature.

Many researchers, have recognized the importance of heredity, race, sex, nutrition, endocrine secretion and disease as factors which influence bone growth and the initial appearance of ossification centers. The weight of the skeleton is an important factor in the understanding of body composition and of problems in nutrition and disease, as found in the living subject (80). Recently, Trotter determined the weight of the dry, fat-free osseous skeleton of 124 American, white and Negro fetuses of both sexes, ranging in age from 16-44 weeks (81, 82, 83). A significant correlation existed between the weight of the total osseous skeleton and body weight, as well as lengths of osseous diaphyses of humerus and femur; all increased with age. From the regression equations, the weights of the long limb bones were found to result in slightly more reliable estimates of skeletal weight than did bone lengths. Either

weight or length of long bones permitted more reliable estimates of total skeletal weight than did gestational age, birth weight or length of fetus. Significant race, but not sex differences were found for lengths of long limb bones, with bones of Negros being longer than those of whites. The ratio of the length of femur to humerus and of tibia to radius showed sex differences, with female ratios higher than male, but neither race nor sex differences were found for the weight of selected parts of the free limbs or for the total skeleton. This is in contrast to Roche's (84) observation that during the last three months prenatal and at birth ossification is more advanced in the female than the male.

b) Composition and Development

Knowledge of the changes in composition of long bones during development probably dates from the 1925 study by Hammett (85, 89, 90) on the rat femur and humerus. The fundamental change in the composition of a bone during development is a result of an increase in the degree of ossification, accompanied by a fall in the percentage of water. Hammett concluded that the progressive deposition of bone ash during growth is the cause of the displacement of water, and the increment in organic matter plays a relatively insignificant part in the dehydration which occurs with age. This generalization seems to hold for

most species, especially when the composition of the bones is expressed on a fat-free basis.

Table I from Dickerson (86) shows the composition of the whole human femur between 12 to 14 weeks gestation and term. The changes are very clear - the fall in percentage of water and the increase in collagen and bone mineral, as indicated by the calcium and phosphorus content. A rise in the calcium/nitrogen ratio indicates the increase in degree of calcification of the bone. The cleaning of bone samples for analysis takes considerable time and careful precautions are necessary if the percentage of water is to be accurately determined. It has therefore been customary to express the composition of bone tissue on a dry fat-free basis. When this is done, the amounts of organic matrix and mineral bear an inverse relation to each other. No detectable fat has been found in the femora of fetuses up to 28 weeks gestation and at term it amounted to 0.14% (86).

These changes in whole bone represent changes in a composite structure, for a long bone consists of bony tissue, marrow and cartilage, and all of these are changing in composition and relative size. Over this period of development the weight of the epiphyses expressed as a percentage of the weight of the femur was found to fall from 73% to 50%, at the same time the percentage of water in the epiphyses fell and the concentration of collagen and calcium

Table I. Composition of the whole femur of the human fetus[a]

Constituent	Fetal age (weeks)					Term
	12-14	15-16	20-24	25-28	30-34	
Weight of femur (gm)	0.11	0.22	1.96	4.7	9.2	16.6
Fat in fresh bone (gm/100gm)	0	0	0	0	0.15	0.14
	Composition of fresh fat-free bone[b]					
Water	77.8	78.4	72.9	68.4	63.8	63.9
Total N	1.61	1.66	2.01	2.19	2.35	2.71
Collagen N	0.61	0.81	1.11	1.36	1.52	1.67
Ca	2.42	3.47	4.33	5.25	5.63	6.06
P	1.50	1.61	1.97	2.36	2.59	2.84
Ca/N	1.50	2.09	2.18	2.40	2.42	2.24

[a] From Dickerson (86)

[b] In g/100g

increased by a factor of approximately three. The ratio of calcium to nitrogen rose and there was also a considerable increase in the ratio of calcium to phosphorus. Dickerson (86) suggested that the increase was due to a fall in the proportion of phosphate from ester phosphates, a large part of the phosphorus in the bone of the immature fetus being present in this form.

Dickerson has also tabulated the main developmental changes in cortical bone composition, expressed per 100g of dry fat-free solids (Table II). From 12 to 34 weeks the percentage of total N fell and that of collagen N rose, if somewhat irregularily. This proportion of total N accounted for by collagen has been shown to increase at certain stages during development of bone in man, the pig, rat, fowl, but the stage of development at which the rise occurs varies from one species to another. Thus, in the human bone the main increase takes place before 22 weeks gestation and in the pig, before 65 days gestation. In the rat and fowl, on the other hand, the same increase occurs during postnatal growth (87). In the cortex of the human femur, Dickerson (86) observed that collagen accounted for 89-96% of the total N after 9 months of age (88).

The percentage of calcium in the tissue increased until the 34th week and so did the Ca/N ratio. The validity of the Ca/N ratio as a measure of the degree of calcification

Table II. Composition of the cortex of the femur during the fetal life[ab]

Constituent	Fetal Age (Weeks)			Term
	12-14	20-24	30-34	
Total N (gm/100 gm)	5.95	5.25	5.03	5.06
Collagen N (gm/100gm)	2.9	4.05	4.03	4.20
Ca (gm/100gm)	18.9	23.4	24.7	24.6
P (gm/100gm)	9.1	10.5	10.9	10.8
Ratio Ca/N	3.2	4.45	4.9	4.9
Ratio Ca/collagen N	6.5	5.8	6.1	5.8
Ratio Ca/P	2.4	2.2	2.3	2.3

[a] Dry, fat-free bone

[b] From Dickerson (86)

of bone depends upon the cleanliness of the samples of bone analysed (91). Bone begins to be laid down in the cartilage model of the human femur at about eight weeks gestation. Before this the Ca/N ratio may be considered to be practically nil (92). By 12 weeks, the ratio had increased to 3.0 and by 22 weeks gestation it had increased to 4.5 (86). These changes in the degree of calcification of the human bone during fetal development and also the relative degree of calcification of bone from full term babies and that from adults agree well with the findings of Wallgren (57), based on biophysical methods.

Since the crystals of bone mineral are mainly laid down in association with the collagen fibrils, the Ca/collagen ratio gives a measure of the degree of saturation of the collagen fibrils. As seen from Table II, this ratio changed very little during growth in humans. This is in agreement with the currently accepted view that the collagen fibrils are rapidly mineralized to about 80% saturation soon after they are laid down (93).

Ca/P ratio remained constant with age when expressed per 100g of dry fat-free solids. This confirms the earlier observation of Swanson and Iob (94, 95) who found also that the concentration of magnesium, sodium and chloride in bone ash decreased with fetal development. This would imply a rise in the Ca/Mg and Ca/Na ratios. Various workers (85, 96)

have obtained different results for Ca/Na ratios depending on the species and stage of development, for the following reasons. Sodium is found in the bone in extracellular fluids, in the hydrated layer of bone crystals, and in the bone crystals themselves. The sodium of the bone crystals, and also the magnesium, are thought to be absorbed on the crystal surfaces, (97, 98, 99). With development, the percentage of extracellular fluid in bone drops, thus the sodium in this fraction also falls. At the same time the bone is becoming progressively calcified and the sodium associated with the crystals increases. Finally, as bone crystals enlarge in size there is correspondingly less sodium on their surface.

The citrate of fetal bone increases progressively with development according to one author (100) and falls according to another (101). McCance et al (96) found a large but temporary rise in the concentration of citric acid 4 weeks after birth in the cortical bone of pigs. The concentration of fluorine in human bone has been found to increase during prenatal (102) and postnatal (103) growth. Its rate of deposition is more rapid in those areas of bone where the metabolic activity is greatest. The value found in adult bones is to some extent dependent on the fluoride content of the drinking water but even where there is none in the water there may be an appreciable intake of the element, because tea is an important source.

Strontium in fetal bones has been estimated as 0.016% of the bone ash, whereas the mean value for all the postnatal samples was 0.022% (104).

The membrane bones of the skull develop rather differently from the long bones, as indicated both by microradiography (105) and chemical analysis (106). In man, McDonald (106) found a small increase in the concentration of calcium, a larger increase in that of carbonate, and no change in the concentration of phosphorus or collagen per unit weight of dry bone between 28 weeks gestation and term. He suggested that the apparent increase in the proportion of bone mineral present in the form of carbonate might be part of the 'hardening' of the fetal head associated with maturity.

B. Maternal Nutrition and Fetal Growth and Development

1. Role of Nutrition

The continued normal growth of the fetus throughout pregnancy, assuming genetic potential and optimum environment, depends upon the integrated development of maternal and fetal placental circulation, with an adequate concentration of nutrients in maternal blood and an adequate area of normal placental membrane for fetal transfer. Poor fetal growth could in theory result from a) conditions affecting the nutrient content of the maternal blood or its supply to the placenta; b) poor development of,

damage to, or specific abnormalities of the placental membrane affecting transfer across the placenta, or c) disorders of the fetal placental circulation. Available evidence suggests that fetal nutrition may be impaired at any of these sites (10, 107).

Evidence for the role of nutrition in human pregnancy is derived from three general sources: a) records of large population groups with varying socio-economic and health status; b) data from supervised hospital and clinic groups; and c) controlled, prospective studies of patients receiving prescribed diets and/or nutritional supplements, frequently with laboratory observations. These and other pertinent information have been reviewed by Burke (108) and have been considered more recently in the 1970 N.R.C. maternal nutrition study (109, 100).

The rate of growth before birth, like the rate of growth afterwards, depends primarily upon the food supply and upon the ability of the fetus to take in and make use of the food. Widdowson (111) and others (112, 113, 114, 115) have reviewed how the fetus is fed generally, body composition and placental transfer of nutrients.

Controversy still rages over nutritional needs during pregnancy. For example, in a recent letter in the American Journal of Clinical Nutrition, Garn (116) remarks on how little is known about actual calcium requirements in man,

either for bone development, or for skeletal maintenance. During pregnancy, less than 20g calcium is incorporated into the fetal skeleton, assuming the weight of the skeleton at birth to be 100g (81). Therefore, calcium retained as new bone approximates 75mg/day during pregnancy and Garn feels it is doubtful whether absorptive efficiency is then so diminished as to justify an additional allowance of 400mg/day at that time. Armstrong (117) determined blood plasma calcium of women in their ninth month of pregnancy. His data suggest that either a calcium 'pump' operates in the placenta supplying a higher concentration of calcium to the fetal blood supply than is found in the maternal circulation, or that the calcium homeostatic mechanism operates at different levels in maternal and fetal organisms. Widdowson and McCance (118) however, suggest the amounts of calcium, phosphorus and magnesium in the maternal serum are not nearly enough to provide for the developing fetus near term.

Clearly, the precise nature of the maternal fetal relationship is not known. The stores of the maternal tissue act as buffers which prevent deprivation of the developing fetus as long as possible (119). It was assumed until recently that these maternal stores either protect the offspring entirely, premitting delivery of normal young, or that in the case of extreme dietary deficiency the fetus dies in utero. Although there is some truth in the 'all or

none' theory it is not entirely correct since between these two extremes there exists a narrow range in which maternal nutritional deficiency may result in arrest of fetal development without causing death (120, 121). In this case, growth of the fetus may be retarded.

2. Effect on Nutrition on Birth Weight

It appears clear from both animal (122-125) and human data (126-129, 120), that starvation can have deleterious effects on fetal growth, resulting in intra-uterine growth retardation, stillbirths and abortion. The magnitudes of these effects are greater in those species with longer gestation periods and larger term fetuses (130). In instances of mass deprivation, as in time of war, low birth weight infants were frequently reported. Even exposures to slightly reduced dietary intake and quality may affect the fetus (131-141). Dokladah (142) attributed an increase in birth weight in a Czechoslovakian sample over a period of 50 years to an improvement in diet, although other factors may have contributed to this change. Toverud (143) in an analysis of statistics gathered in Norway, found a significant increase in the weight of infants born between August and October. In discussing the possible cause of this difference in weight, she mentioned the increase in sunlight during the summer months, the greater availability of fresh fruits and vegetables and the longer

time the mothers probably spent at rest during the warm months. Toverud was inclined to attribute the seasonal differences in weight in her series to a combination of these factors.

Attempts to relate the protein intake of a population of pregnant women to birth weight indicated that the protein requirements are higher during pregnancy, that the requirement is further elevated during the last trimester and that an intake below 70g protein per day results in a small infant (128). A study of overnutrition (144) revealed that although the birth weights of obese adult women fell within the normal range, the birth weights of infants from obese mothers tended to be somewhat elevated. Thomson (145) also reported an unusually accurate correlation between maternal intake of calories and fetal size. If the maternal intake was below 1800 Cal/day, the mean birth weight was 3.09kg and the incidence of 'prematurity' was 8.5%. If the intake was greater than 3,000 Cal/day, the mean birth weight was 3.3kg and the incidence of 'prematurity' was 1.5%, (the international definition of prematurity: $\leq$ 2,500g at birth). If there is a multiplicity of causes for low birth weight of infants, it would be difficult to explain these data unless there also happened to be an inverse relationship between caloric intake and the incidence of toxemia, mothers who smoke, etc.

Other investigators have reported that there was no significant difference in the diet of women who delivered full term babies and those who had premature infants, but a normal rate of intra-uterine growth can be associated with either full-term or premature infants (146-158). If the food deprivation is severe, the incidence of prematurity may rise. Experiments with rats demonstrated that if starvation was initiated at the midpoint of pregnancy, there was a 40% reduction in weight of the offspring (159). The same experiment repeated with only moderate food deprivation did not reduce fetal or placental weight (160). It has been reported that pregnant rats with only a poor dietary history may deliver low birth weight fetuses but that food deprivation during pregnancy is a more significant factor (124). Although fetal stunting is more severe if food deprivation occurs during the latter part of pregnancy, fetal growth retardation has been reported as early as 90 days when maternal sheep were undernourished during the first half of pregnancy (161). Other experimental techniques that may inadvertently interfere with maternal nutrition and influence birth weight. Pregnant rats subjected to irradiation of the head produced stunted young at term (162). Not only were the fetuses reduced in size but the mother also did not gain a normal amount of weight during her pregnancy. Poor intake and poor maternal nutrition could have contributed directly to fetal loss and fetal

growth retardation.

3. Nutrition and Bone Growth

a) Animal Studies

The normal shape of a bone is the result of a balance between rate of growth in thickness and rate of growth in length. These two processes may be affected to different degrees by changes in the level of nutrition. Experiments with growing animals appear to support this hypothesis. In rats held at birth weight for two weeks by underfeeding, the skeleton continued to grow very slowly while ossification also proceeded slowly (163). The bones of other young animals reared on a maintenance or subsistance diet continued to grow but at a much slower rate than normal (164-168). For example, retarding growth of chickens by underfeeding was found to depress increase in femoral thickness to a greater extent than increase in length (87). Appleton (169) attributed variations in the size of young rabbits of the same age to differences in nutritional background, concluding that the level of nutrition affects both the rate of growth and the rate of bone ossification.

The cortex of the long bones of pigs and cockerels whose growth was greatly retarded for long periods of time by underfeeding was very thin and brittle and the Ca/collagen ratio was significantly higher than in well nourished animals of either the same body weight or same chronological

age, (170). The structure of the cortex of the bones of these animals was also abnormal and the chemical findings were possibly related to this. The abnormality in the composition of the cortex induced by underfeeding was completely masked when the composition of the whole bone was considered, for in both species the Ca/collagen ratio was the same as or lower than in normal bones of the same age.

Other animal experiments have been conducted in which food intake was increased materially during the first few days of life (171). Rats in this group grew much faster throughout their whole growth period than their littermates so that they became larger adults and remained large for the rest of their life. Using alizarin staining, weight and length measurements and determination of the composition of rat femur, Dickerson (172) studied this effect of accelerated growth on skeletal development. He found that faster growth rate affected maturation to different extents although body length was always proportional to body weight. Earlier appearance and fusion of the epiphyses was seen. Long bones were short for body weight and length in rapidly growing animals, suggesting that the skeleton of a highly nourished animal is in a less advanced state of ossification than the skeleton of a poorly nourished animal which has finally reached the same size after a longer period of growth. Thus the morphologically immature femora of the accelerated rats may be due to a high plane of nutrition having increased

growth in thickness to a greater extent than growth in length.

Hammond (173) and his associates have carried out a number of investigations on the effect of different levels of nutrition upon the skeleton of the pig and sheep. In one of these, Wallace (174) showed that the skeletal development of lambs depends on the level of nutrition of the mother during the last six weeks of pregnancy and on the number of lambs carried by the ewe. The skeletons of lambs born of mothers which had been maintained on a high level of nutrition, and more especially if they were singletons, were in a more advanced state of development than those of twins or triplets and of lambs born of ewes reared on a low plane of nutrition. Ossification of bones was also found to be more advanced with the former group. This study suggested that size of skeleton was a better reference for skeletal development than was body weight.

The calcium content of the diet is of prime importance to the growth and development of the animal skeleton. The proportion of calcium in the newborn rat seemed to be unaffected by only reducing the calcium intake of the mother during pregnancy (175). The calcium/phosphate ratio of the diet appears to be the critical factor in this species. Henry and Kon (176) showed, in rats, that low concentration of phosphorus in the diet reduced the retention of calcium.

Warkany (177) noted that when rats raised on rachitogenic diets were bred, alizarin staining of the young indicated that 57 out of 164 had multiple skeletal deformities, compared to no abnormalities in the control group. Many paired animal experiments have showed skeletal differences when young were raised on varying levels of calcium. With increasing increments of dietary calcium, female rats had a longer life span and were able to rear more young, sturdier offspring. It was also found that animals which had received ample food calcium but were stunted in growth because of other dietary deficiencies (vitamin A, thiamine, protein) had higher concentration of skeletal calcium than did normal rats of the same age (178).

Marginal protein deficiency during the reproductive cycle has resulted in lower bone growth potential in the skulls of newborn rats (179) and in severly depressed endochondral bone formation in monkeys (180). When growing rats are fed diets containing only trace amounts of magnesium or sodium the concentration of that mineral in the bones falls (181). Even deficiencies of trace elements such as zinc and manganese has produced skeletal retardation (4).

From similar kinds of studies, theories have been proposed regarding the specific effect of dietary manipulation of endochondral ossification (182). Deficiencies of calcium, phosphorus and Vitamin D will impair erosion of

hypertrophic cartilage cells and thus retard calcification. Maternal hypervitaminosis D_2 has resulted in smaller diaphyses of fetal bones and has produced alteration in ossification with the appearance of pathological types of cartilage cells in the epiphyseal area. Uncontrolled osteoblast activity causes overgrowth of bone in Vitamin A deficiency, whereas in hypervitaminosis A, bone formation ceases and fractures occur from depressed osteoblastic acitivity. In riboflavin deficiency there is a gradual cessation of calcification, the primary spongeosa disappears, the epiphyseal cartilage narrows and is finally sealed off with bone. Pantothenate and pyridoxime deficiencies, protein deficiency and inanition show the above effects also, presumably due to interference with matrix formation rather than with cessation of calcification. Ascorbic acid deficiency interferes with the activity of osteoblasts, which then revert back to fibroblast-like cells; bone weakness and fractures result. Vitamin E does not appear to be directly involved in endochondral ossification.

b) Human Studies

Although there is a considerable amount of information available concerning the role of maternal nutrition on the development of the fetus in experimental animals, the relevance of these data to the problem of human fetal deprivation, particularily in the area of skeletal develop-

ment, is hard to assess. Usually the dietary deficiency or deficiencies utilized are gross to ensure major defects; the gestation periods are very different from that of humans; most laboratory animals are highly polytocous; and finally the intra-uterine development is very different from that in man, in that the fetal organism is more highly differentiated at birth compared to man.

A limited number of studies of newborn infants, and considerably more studies of growing children, have related nutrition to skeletal development. Figure 2 shows the relationship that Stuart (183) obtained between maternal diet and osseous development of the hand, knee and foot, based on X-Rays taken at birth. In this study, "poor diet" could be strongly correlated with retardation in the infant's osseous development. The difference between a "good" or "excellent" maternal diet and a "fair" diet was not as striking, although there were more retarded infants in the "fair" maternal diet group than in the other groups. It was obvious that few infants were advanced and many were retarded in the "very poor" diet group.

Stuart found an even stronger relationship when protein content of the maternal diet was correlated with osseous development at birth. In the "excellent" protein diet group, 57% of the infants were advanced and 14% were retarded, whereas in the "poor" protein group, none were

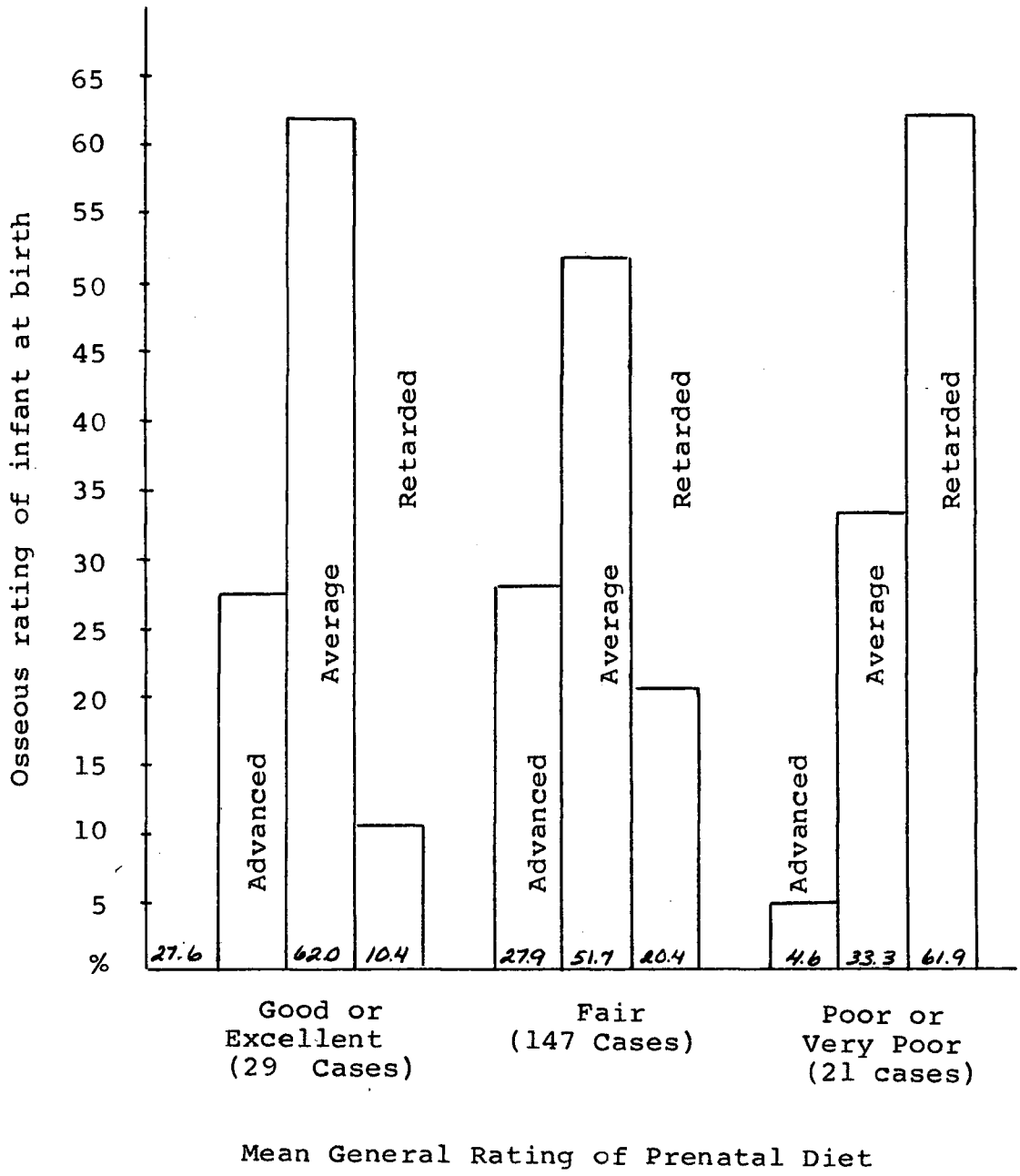


Figure 2. Relation of osseous development of living, full-term infants at birth to their mothers' diets during pregnancy (183)

advanced and 71% of the newborns were retarded in osseous development. A somewhat less marked relationship was found when maternal dietary calcium was considered: "excellent" calcium diets giving 32% advanced with 23% retarded, and "poor" calcium diets giving 6% advanced and 64% retarded.

Stuart (183) found a similar relationship between the calcification of teeth before eruption and quality of the diet during pregnancy. This reinforced Berk's conclusion (184) that an adequate prenatal diet seemed to be an essential factor in calcification of a child's teeth during the first ten months of life. Massler (185), however, in his discussion of prenatal calcification of teeth commented that almost perfect calcification of certain tissues before birth was not surprising - the fetus or embryo is a parasite deriving all its nutrients from the mother and drawing on her calcium reserves in the bone where necessary. Thus only severe deficiency in the mother could affect tissues calcified before birth. Generally, the "parasite" concept is not accepted.

The calcification of the tibia of newborn infants was found to be unaffected even when the mothers were only 14-17 years old and were probably calcifying their own bones (186). However, the significance of this finding must be questioned as maternal calcium intake was not determined. Individual bones may differ in the susepti-

bility to a low calcium intake by the mother. Toverud and Toverud (187) reported that the percentage of calcium in the parietal bones and ribs of newborn infants was lower when the diet of the mother contained practically no milk and was therefore very low in calcium. The degree of calcification of the infant's skull at birth may be influenced by prenatal factors. Boder (188) specifically implicated maternal exposure to sun and supplemental administration of dicalcium phosphate together with Vitamin D. A review of literature on prenatal rickets indicated that maternal health, diet, frequent pregnancy, and lack of exposure to sunshine may be contributing factors which exert an influence on the development of rickets in very young infants (189). Toverud (190) studied the etiology of congenital osteoporosis, finding that poor calcification of fetal bone correlated with the negative calcium and phosphorus balance common during the last 2 to 3 months of pregnancy. Cockburn (191) in exploring some biochemical aspects of intra-uterine growth retardation, reported that plasma calcium was singificantly reduced and inorganic phosphate significantly increased in umbilical vein plasma of low birth weight infants.

Sontag (192) attempted to clarify the relationship between certain maternal conditions during pregnancy and the state of well-being of the fetus at birth, as measured by length, weight and blood calcium and development of bone.

No correlation was shown between the following sets of factors: (a) the calcium content of the serum of the mother and the total fetal epiphseal area; (b) the length of infant at birth and the fetal epiphyseal area; (c) the adequacy of the maternal diet and the fetal epiphyseal area; (d) the adequacy of the maternal diet and the calcium content of the serum in the cord; (e) the amount of calcium in the maternal diet and the calcium content of the serum in the cord; (f) the mother's gain in weight and the weight at birth; (g) the mother's caloric intake and the weight at birth; (h) the mother's protein intake and the fetal epiphyseal area; (j) the amount of fat in the mother's diet and the fetal epiphyseal area; (k) the amount of calcium in the mother's diet and the fetal epiphyseal area; (l) the amount of phosphorus in the mother's diet and the fetal epiphyseal area; (m) the menstrual age of the fetus and the total epiphyseal area; (n) the diet of the mother and the home conditions. From what was known about the seeming independence of the growth of new bone (or at least the transformation of cartilage into osteod tissue) and rickets, Sontag felt that the intake of calcium, phosphorus, and vitamin D was probably more important in the determination of bone density than in bone growth. Although an annotated bibliography (193) on bone density has recently been published, no literature is available on the density of human fetal bones and its relation to maternal nutrition.

Tompkins (194) has related epiphyseal maturation in the newborn to maternal nutritional status. Hospitalized pregnant women were given varying amounts of nutritional supplements (protein, vitamins, minerals) during the final 16 weeks of pregnancy. The area of ossification was measured from radiographs of the newborns' heels and knees. Individual differences were found in the time of formation of the three centers studied. Negros developed earlier than whites, and the female, regardless of race, was more advanced than the male. Among patients who took supplements there was a significant probability that the tibial epiphyseal center of the knee would be present in female infants. These supplements did not alter the time of appearance of the epiphyseal center. Interestingly, Tompkins' population was not experiencing any serious nutritional deficiencies. The patients who did not receive supplements reported a daily diet in the last half of pregnancy which included, on the average, 76g protein and 860 $\frac{mg}{\lambda}$ calcium.

Other researchers have reported that epiphyseal development during the intra-uterine period was markedly delayed in fetal malnutritional syndrome. Femoral and tibial epiphyses were absent in a higher percentage of the undernourished group than the controls, and even when present, the centers in the malmourished infants were smaller (195). Postnatal bone growth of infants with fetal growth retardation has also been investigated (196).

Infants with birth weights lower than the tenth percentile for gestational age had shorter fibulas and retarded development of the epiphyses at the knee when compared to infants with normal weight for gestational age. The majority of infants small at birth grew at a normal rate during neonatal life (197). Unfortunately, conclusions cannot be drawn from these experiments unless poor nutritional status of the mother had been clearly differentiated from placental dysfunction (198).

In children there is a definite sequence as well as date of appearance for secondary centers of ossification, but this schedule may be interrupted or retarded by metabolic or constitutional distrubances. Weight, body maturity and even mental development may show irregularities in their progress (199). Epiphyseal rating is the earliest and frequently the only indicator of disturbances in growth and is more delicate than measures of weight or height (200). Epiphyseal rating may be influenced by the availability of minerals and Vitamin D in the diet and the general level of nutrition (201). Studies on the development of epiphyseal ossification in children with kwashiorkor (202) and in malnourished German children (203) have shown that nutrition may alter the rate at which a bone develops, thus masking the usual effects of chronological age. Although Dickerson and John (204) postulated a deficiency of protein in the bone marrow, there were no differences in the composition

of the femur as a whole, or of the epiphyses or the cortex, that could be attributed specifically to kwashiorkor or marasmus.

I N T R O D U C T I O N

The nutritional process can be regarded as a continuum that begins with conception and in which emergence to extra-uterine life is merely a transition rather than a beginning. Intuitively, the nutritional status of the pregnant woman could affect skeletal growth and development of the fetus.

Growth at the end of the fetal period has been assessed by such criteria as birth weight and length, foot length, circumference of the head, chest, abdomen and thigh, and skin-fold thicknesses (17). Maternal nutrition during pregnancy is but one factor that has been shown to affect certain birth size parameters. For example, there is some evidence that development of secondary ossification centers, as determined at birth, is related to maternal diet (183, 194, 195).

By contrast, the available information regarding growth during the pre-birth period has been obtained largely from premature births or spontaneous abortions. In each case the pregnancy was abnormal because it failed to reach term. Dickerson and colleagues (86, 106) have described bone composition of fetuses 12 weeks to birth. Trotter (81-83) has recently studied bone length, weight and density of the human fetus. Gruenwald (49-51) has related socio-economic factors to fetal birth weight, 24

weeks to term. Whereas the contribution of each work is impressive, no correlation has been made between these various studies. It is not known whether the fetuses studied by Dickerson, Trotter and Gruenwald were representative of normal growth. In addition, no research relating maternal diet to growth of the human fetus, 8-20 weeks old, is available.

The purpose of this project is two-fold: (a) to define certain parameters of skeletal growth and development in the normal human fetus, and (b) to correlate certain maternal factors with these fetal parameters. It is hoped that the fetal model developed will give direction to further research in this area.

Normal human fetuses were made available for this project through the cooperation of Dr. Betty Poland of the Department of Obstetrics and Division of Human Genetics, U.B.C. Length, weight and external measurements analogous to those taken on newborns were recorded for the intact fetus. The humerus and femur were chosen as representative models of endochondral bone growth and, thus, of skeletal growth. Because most of the research has been conducted on the femur, the humerus was included in this study to compare growth rate in the fetal arm and leg. Both bones were weighed, measured, and radiographed to provide physical indices of bone growth. The bones were assayed for certain minerals and collagen to provide biochemical

indices of bone development. Three criteria of fetal growth were therefore available; whole fetal measurements, physical data and biochemical data of long bones.

Maternal factors other than nutrition have been related to fetal growth and development (20-49). To provide perspective between nutritional and non-nutritional factors, selected medical information, growth data, and socio-economic scores were also collected and correlated with fetal parameters.

M A T E R I A L S A N D M E T H O D S

A. Fetal Studies

Seventy-six human fetuses of varying ages and sex were collected immediately following therapeutic abortion via hysterotomy. Crown-rump length was measured and developmental age calculated from a modification of Streeter's (205) graph (Table XI). The umbilical cord was cut at the naval and the intact fetus was weighed. Head circumference, sex and limb measurements were recorded. Where possible, length of cord and weight of placenta were noted. The fetus was dissected and eviscerated using standard autopsy procedure at Vancouver General Hospital. The right arm and leg were carefully removed at the clavicle and the pelvic joints, respectively, for biochemical analysis. The remainder of the fetus was placed in 10% buffered formalin, for later radiological study. The V. G. H. Pathology Lab examined all placentas for lesions. Abnormal fetuses, detected either by autopsy or by placental histology, were excluded from this study.

A photographic study of the following fetal materials and methods has been included in Appendix 1 (Plates 1-9). Appendix 1 also contains all tables, figures and forms relating to methods.

1. Long Bone Studies - Physical

Flesh and tendons were carefully cut from limb bones of the right arm and leg. The elbow and knee joints were teased apart. Humerus, radius, ulna, femur, tibia, fibula were washed by water pressure, then wiped thoroughly to remove the periosteum. The length of the fresh bone including cartilage was recorded. A Metler Analytic Balance was used to weight the individual bones to 0.01 mg. All the bones were dried to constant weight at 105°C. Mean percent dry matter per long bones per fetus was calculated to provide an index of skeletal weight. The water content (% fresh bone) of femur and humerus was found by subtraction.

Fat was extracted from each fetal bone using the Goldfisch Fat Extraction Apparatus (206). The bones were then dried to constant fat-free weight. The difference between dry weight and fat-free weight was calculated and expressed as a percentage.

2. Long Bone Studies - Biochemical

Sixteen fetuses were placed in formalin before dissection. Because formalin treatment impairs biochemical analysis, bone composition of these specimens was not considered. A total of sixty femora and humeri were analysed individually. Samples of bone powders weighing 50mg or whole bone fragments in the case of those not large enough for powdering, were heated with 1ml 6N hydrochloric acid in sealed tubes in a 100°C block heater for 48 hours. After acid hydrolysis, the mixture was neutralized with 1ml 6N potassium hydroxide. Sufficient HCl was then pipetted to dissolve the calcium phosphate precipitate. After making up to volume with deionized-distilled water, samples of the solution were taken for estimation of calcium and magnesium by atomic absorption (207), sodium by flame emission (208), and inorganic phosphorus by a colorimetric method (209). The percentage of hydroxyproline estimated by Neuman and Logans procedure (210) with the modification suggested by Leach (211) was converted into percentage of collagen on the assumption that human collagen contains 14.1% hydroxyproline (212). All values were expressed as g/100g dry fat-free bone.

3. Long Bone Studies - Radiological

The eviscerated fetus with right arm and leg removed was fixed in formalin for 2 to 7 days (Table XII). Following O'Rahilly's method (72) of silver radiography, the fetus was immersed in a 0.5% aqueous solution of silver nitrate for a period of 2 to 11 days depending on the length of the specimen (Table XIII). The fetus was then rinsed, dried thoroughly and pinned flat, dorsal side down, using wooden toothpicks, on a styrofoam slab. All 76 specimens underwent this treatment.

A 10ma portable roentgen unit was employed. The fetuses were radiographed on non-screen film at 58kv, 10-40mas (Table XIV) and a target-film distance of 24". The X-Rays were processed manually.

The radiographs were illuminated on a screen and, using a millimeter eyepiece with eight-fold magnification, (Flubacher & Co., Horgen, Switzerland) length and width of ossified bone shafts were measured according to Figure 6.

B. Maternal Studies

1. Medical History

The patient's history was taken by Dr. Poland either preceding the therapeutic abortion or within 24 hours thereafter. Information included age of the mother and father, number of children (parity), number of pregnancies including the present one (gravidity), previous abortions or stillbirths, ethnic origin, past medical, obstetric and family history, method of birth control used, and prenatal factors. Gestational age or duration of pregnancy, was calculated by adding 14 days to the date of the last menstrual period and subtracting this estimation of conception from the date of abortion.

The reason for the present abortion and the method of abortion were recorded. The majority of indications involved psychiatric reasons but three normal fetuses from spontaneous abortions were included.

2. Dietary History

Dietary information was obtained from an interview at the patient's bedside three to five days after the abortion. A standard procedure was followed (Form 1), based on short form dietary histories presented in the literature (213-215). First a daily dietary pattern was obtained by asking the patient what she usually ate during

the course of a day throughout her pregnancy. A more precise indication of maternal diet was obtained from a food frequency question. The type or form and amount of food consumed was recorded, and the patient chose the time period (i.e. day, week, month). The patient was then asked specific food likes and dislikes to validate the preceding questions. Any other relevant information from the 15 minute interview was recorded under general comments. This could involve family preferences, previous nutritional status, difference between pregnant and non-pregnant nutrition, any history of nutritional ailments, following of fad diet or very erratic eating habits, and comments concerning the general authenticity of the history.

Maternal nutrition was assessed using Crump's rating (45) which was based on previous studies (115) and on Recommended Daily Allowances (216, 217). Number of servings per week for each of 6 food groups was calculated from interview data and expressed in four possible ways; (Form 2)

(a) Total Nutrition Score (0-133); sum of number of servings per week across all food groups.

(b) Weighted Nutrition Score (0-30); each food group was assigned a maximum value of 5 and response scaled accordingly

(c) Nutrition Index (0-5); based on Weighted

Score divided by number of food groups involved

(d) Protein Score (0-40); sum of number of servings per week in milk and eggs, meat, fish, cheese food groups.

Comments concerning the general adequacy of the maternal diet in Vitamin D, calcium, protein and iron, based on types and amounts of food eaten, were noted on this form.

3. Personal History

Following the dietary history the patient was usually relaxed enough to answer more personal questions. Age, weight, height, ethnic origin, occupation and grade of school completed were recorded for both the mother and father of the fetus. In addition, birth weight of mother was sought. Information which could not or would not be given by the patient was obtained from hospital records where possible.

A short form socio-economic index was calculated using Crump's rating (23), in which the occupation of father, education of mother and father and marital status of mother were considered. Information was coded as total score (0-72) and as Socio-economic Group (1-4) according to the outline (Form 3).

C. Statistical Analysis

Whole fetal measurements, ossification and biochemical data of femora and humeri, nutritional, medical, growth and socio-economic information from the mother were coded for analysis on U.B.C.'s IBM 360/67 computer. Means, standard deviations, degrees of freedom and simple correlation matrices were generated. Simple and multiple regression analysis was performed on the whole range of data for lines of least squares, coefficients of determination and F probabilities. This procedure was undertaken to discover which variables best predicted other variables. The Coefficient of Correlation (r) squared is the Coefficient of Determination (R^2). Because R^2 gives the proportion or percentage of the variance shared by the two variables, this value was considered a more useful means of expression. The closer R^2 is to 1.0, the better the fit is on the regression line.

Each variable was then classified according to age group of fetus (9-10, 11-12, 13-14, 15-16, over 16 weeks developmental age). Two week age groups were chosen to allow comparison with Dickerson's data (86). Analysis of variance was executed along with Duncan's New Multiple Range Test at the 5% level to test the significance of each group mean.

R E S U L T S

A. Fetal Data

Fetal data were grouped according to developmental age into five age periods of two weeks each. The number of specimens in each group (total 76 specimens) is presented in Figure 3. Experimental error, and the fact that only 60 femora and humeri were analysed, reduces the total sample size. The smallest variable size is 57.

Results of analysis of variance are expressed in Tables III and IV. Total number of observations, means of each age group, and units of expression are given. F probability indicates the liklihood of obtaining an age group effect for that variable by chance alone. If a significant F was found at the 5% level, Duncan's New Multiple Range Test (218) was executed to determine which means were significantly different from each other. Duncan's Test adjusts the "least significant difference" t-test so that the number of means in comparison are included in the calculation. Means sharing the same letter are not significantly different from each other; means assigned a different letter are significantly different at the 5% level.

Figure 3. Histogram of fetal age groups

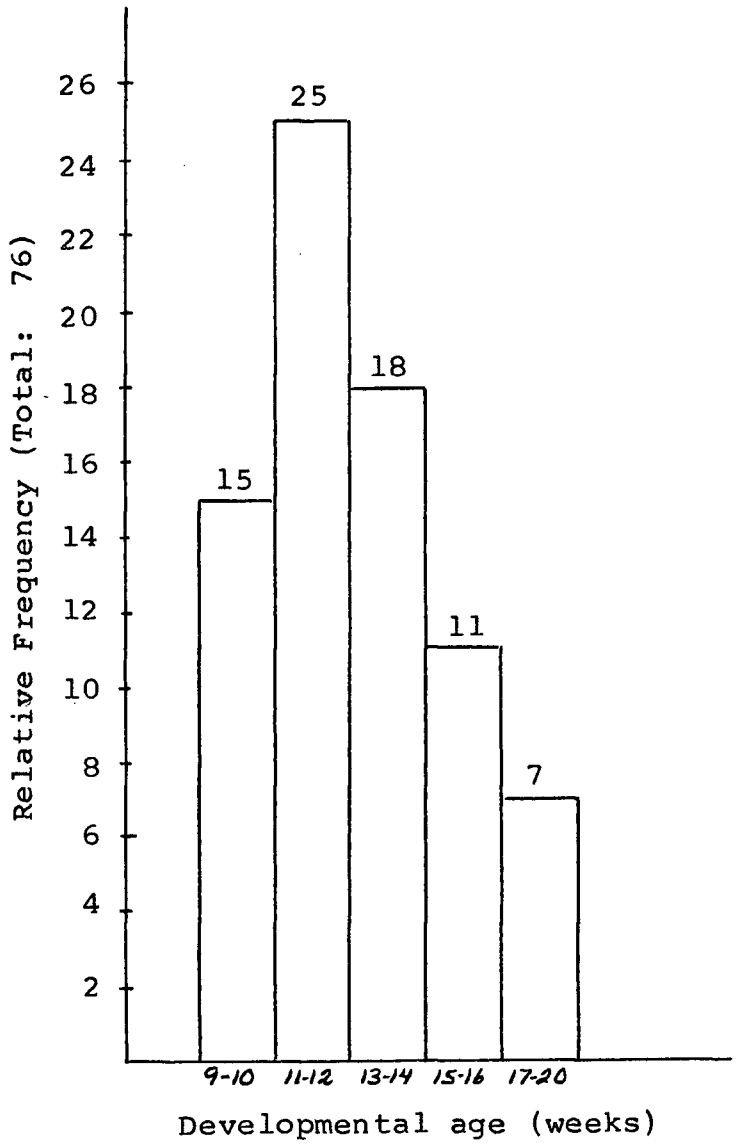


Table III. Fetal and Long Bone Growth related to Developmental Age

Variables	n	Unit	Age of Fetus (weeks) 9-10	11-12	13-14	15-16	16	F Prob.
Crown-rump length	76	mm	61.00a	87.04b	115.44c	139.91d	171.71e	0.0
Developmental age	76	days	69.87a	83.76b	97.94c	110.91d	131.71e	0.0
Gestational age	74	days	69.60a	86.50b	102.06c	109.09c	132.86d	0.0
Fetal weight	67	g	14.20a	41.03b	94.55c	169.77d	340.96e	0.0
Skeletal index	58	%	16.58a	20.65b	25.11c	29.35d	31.13d	0.0
F-dry weight	60	mg	3.40a	17.20a	75.09b	181.21c	384.06d	0.0
H-dry weight	60	mg	4.20a	18.18b	64.20c	127.34d	252.19e	0.0
F-water content	58	%	86.16a	83.61b	78.78c	75.73d	73.49d	0.0
H-water content	58	%	83.57a	79.83b	75.62c	71.80d	69.88d	0.0
F-fresh length	57	mm	13.32a	21.93b	31.29c	39.43d	48.50e	0.0
H-fresh length	57	mm	13.11a	20.88b	28.92c	3.621d	43.50e	0.0
F-ossification	76	mm	5.51a	11.52b	18.99c	25.39d	32.50e	0.0
H-ossification	76	mm	6.23a	12.06b	19.12c	25.28d	32.01e	0.0

F = femur, H = humerus

0.0 indicates F probability $< 10^{-8}$

If F probability ≤ 0.05 Duncan's New Multiple Range Test was performed at the 5% level. Means sharing the same letter are not significantly different from each other; means assigned a different letter are significantly different at the 5% level.

Table IV. Composition of Fetal Long Bones According to Developmental Age

Variable	Unit	Age of Fetus (weeks) 9-10	11-12	13-14	15-16	16	F prob.
F-collagen	g/100g dry fat-free bone	18.23	21.18	20.86	21.32	21.36	0.1285
H-collagen		16.72a	20.95b	21.55b	21.60b	20.86b	0.0013
F-calcium		10.26a	13.11b	16.60c	17.62cd	19.90d	0.0
H-calcium		9.72a	14.80b	17.57c	17.84c	19.81c	0.0
F-phosphorus		6.09a	6.51a	7.77b	8.80b	8.87b	0.0000
H-phosphorus		5.75a	7.23b	7.88bc	8.18bc	8.80c	0.0000
F-magnesium		0.57	0.52	0.52	0.47	0.51	0.2584
H-magnesium		0.58	0.55	0.55	0.48	0.50	0.3811
F-sodium		4.72a	1.35b	0.98b	1.06b	0.98b	0.0000
H-sodium		4.12a	1.06b	0.91b	1.09b	0.86b	0.0
F-calcium/collagen	ratio	0.56a	0.64a	0.79b	0.83b	0.93b	0.0000
H-calcium/collagen	ratio	0.57a	0.72b	0.82bc	0.83bc	0.95c	0.0000
F-Ca/P	ratio	1.68a	2.02b	2.15b	2.18b	2.25b	0.0000
H-Ca/P	ratio	1.68a	2.05b	2.22c	2.18bc	2.25c	0.0

n = 59 for each variable

F = femur, H = humerus

0.0 signifies F prob. $< 10^{-8}$
0.0000 signifies $10^{-8} <$ F prob. $< 10^{-5}$

If F probability ≤ 0.05 Duncan's New Multiple Range Test was performed at the 5% level. Means sharing the same letter are not significantly different from each other; means assigned a different letter are significantly different at the 5% level.

1. Whole fetus

Means, standard deviations and sample sizes of the whole range of variables computed are presented in Table V. Sixty-eight percent (mean $\pm$ 1 standard deviation) of the specimens collected were 70-138mm in length from crown to rump and therefore had a developmental age of 74-112 days or about 11-16 weeks.

Table V. Means and standard deviations of whole fetal variables

	Unit	Sample Size	Mean	Standard Deviation
C. R. Length	mm	76	104.1	34.15
Developmental Age	days	76	92.72	18.55
Gestational Age	days	74	94.39	22.57
Fetal Weight	g	67	89.40	91.32
Head Circumference	mm	67	105.4	36.32
Foot Length	mm	76	18.34	8.057
Skeletal Index	%	58	22.29	5.423
Sex	1= ♀ 2= ♂	76	1.553	0.5005

From Table III, as developmental age of the fetuses increased, CR length, gestational age, weight, head circumference, foot length and skeletal index increased proportionately. Developmental age was best predicted by fetal length, as would be expected from the method of determination (R^2 = 0.995). Because of extrapolation from the graph via a table, this value was not 1.00. Head circumference (R^2 = 0.96), foot length (R^2 = 0.95), weight (R^2 = 0.85), and skeletal index (R^2 = 0.77) all predicted developmental

age with an F probability of $<10^{-8}$. As seen in Table III, each of the above variables separated cleanly into the 5 age periods, except skeletal index which tapered off after 16 weeks. Whereas gestational age was still a significant predictor of developmental age, scatter reduced the Coefficient of Determination to 0.67, and only 4 distinct groups were found in Table III. Scattergrams of these variables are presented in Appendix 2 (Exhibits 1-6). The complete regression data have been deposited with the School of Home Economics and are available upon request.

Gestational age itself is predicted by foot length ($R^2 = 0.70$), C.R. length of fetus ($R^2 = 0.67$), head circumference ($R^2 = 0.67$), weight ($R^2 = 0.58$) and skeletal index ($R^2 = 0.49$). All the above relationships were significant at $p = <10^{-4}$ but greater scatter was evident compared to similar regression against developmental age (Exhibits 7-11).

More male specimens than female specimens were collected (ratio 42:34). Because sample size was small and sex was unevenly distributed throughout the age range, performing separate regression analysis on each group would not have been meaningful.

2. Long Bones

Means, standard deviations and sample sizes for the variables computed are presented in Table VI. Generally, data from the femora and humeri were comparable, with the suggestion that the humerus is slightly more developed for its dry weight than is the femur.

Table VI. Means and standard deviations of long bone variables

Variables	Unit	n	femur		humerus	
			Mean	Stand. Deviat.	Mean	Stand. Deviat.
Dry weight	mg	60	69.15	104.4	52.53	68.03
Water content	%	58	81.66	4.55	78.28	4.99
Fresh length	mm	57	25.80	10.97	24.13	9.54
Ossification	mm	76	16.04	8.65	16.33	8.24
Collagen	g/100g dry bone	59	20.44	3.60	20.14	3.68
calcium		59	14.14	3.68	14.86	4.03
phosphorus		59	7.03	1.29	7.23	1.45
magnesium		59	0.52	0.10	0.55	0.11
sodium		59	2.02	1.90	1.75	1.67
Ca/collagen	ratio	59	0.70	0.16	0.73	0.16
Ca/P	ratio	59	2.00	0.27	2.03	0.26

The constant dry weight of both femora and humeri increased with developmental age (Exhibits 12-13). The non-linear relationship may explain why the femoral data separated into only 4 significant age groups, suggesting a lag period followed by a proportionately larger deposition of mineral and organic matter after 12 weeks. However each of the 5 group means for humeral dry weight were significant.

The weight of the intact fetus was the best predictor of the dry weight of both bones (femur R^2 = 0.98, humerus R^2 = 0.99). Dry weight of the corresponding bone, developmental age of fetus, ossification of the bone, fetal length and length of the fresh bone were also good predictors of a bone's dry weight (R^2 =0.80). Whereas gestational age predicted bone weight with $p < 10^{-4}$, scatter around the regression line was greatly increased (R^2 = 0.54). Biochemical variables were even poorer predictors of the dry weight of both femura and humeri.

An inverse relationship was found between water content of femora and humeri, developmental age and all other variables computed (Exhibits 14-15). Four significant age groups were found suggesting that as the fetus ages, organic and mineral material replaces the water in fetal bones, reaching a plateau at 15 weeks. The water content of the corresponding bone, length and ossification of bone, fetal length and developmental age best predicted the percentage of water in both bones (R^2 = 0.90). The calcium content of the bone resulted in less scatter when plotted against water content than did gestational age against water content.

The lengths of the fresh long bones increased proportionately with developmental age and could be separated into 5 distinct age groups. The lengths of the

femora and humeri were best predicted by the ossification of the bone (R^2 = 0.99) and also by the wet length of the corresponding bone. Fetal length, developmental age, weight of fetus, dry weight and water content of the bone were also good predictors, in that order. Again, calcium content of the femora and humeri was the only biochemical variable with little scatter and was more useful in predicting bone length than was gestational age.

As developmental age increased so did length of ossification in both bones; this effect was significant in each of the 5 age groups (Exhibits 18-19). Indeed, this variable was the best long bone predictor of fetal age (R^2 = 0.96). Length of ossification of the humerus best predicted ossification length of the femur and vice versa. Fresh bone lengths predicted ossification nearly as well (R^2 = 0.99), followed by C.R. length, developmental age, weight of fetus, dry weight and water content of the bone. Gestational age and calcium content showed a good correlation with bone ossification (R^2 = 0.68).

A non-linear effect was seen when collagen content of femora and humeri was plotted against developmental age (Exhibits 20-21). As result when fetal age was grouped into two-week periods little significant difference was found. Group means for femoral collagen ranged between 18.23 - 21.36 g/100g dry bone, with a 12.85% probability of

a significant difference between age groups. However, collagen in the humeri of fetuses 9-10 weeks old was significantly less than that found in fetuses 11-20 weeks old. This suggests that the total amount of collagen increases more rapidly with age in younger fetuses and deposition slows after ten weeks. There were no highly significant predictors of collagen in fetal bone. Femoral collagen was best predicted by humeral collagen (R^2 = 0.50) whereas humeral phosphate best predicted humeral collagen (R^2 = 0.56).

There was a linear relationship (Exhibits 22-23) between developmental age of fetus and femoral calcium (R^2 = 0.65) and humeral calcium (R^2 = 0.55). When analysed according to fetal age group the amount of calcium in the humerus was significantly different in 9-10 week, 11-12 week and 13-20 week fetuses. Femoral calcium showed a significant group effect into 4 ages, with some overlap. Group means suggest a greater increase in calcium deposition in the humerus during the 11-12 week period than in the femur, followed by a plateau from 13-20 weeks. Deposition appears more gradual in the femur; approximately the same final value per 100g dry bone was seen in both femora and humeri. Individual variation and experimental error cannot be ruled out in differences of this magnitude. The phosphorus content of the bone best predicted its calcium content (femur R^2 = 0.76, humerus R^2 = 0.85). In each bone, calcium was the biochemical variable that best predicted physical variables in that bone.

A non-linear effect was suggested by the scattergram of phosphorus content plotted against developmental age (Exhibits 24-25). There was a significant grouping effect into 2 fetal age periods for femoral phosphorus and into 3 for the humeral values, although some overlap was seen. Humeral P was significantly lower than femoral P in the 9-10 week age group but was significantly higher at 11-12 weeks and about the same per 100g dry bone in the remaining age groups. Correlation between femoral and humeral P values produced $R^2 = 0.69$. Humeral P had only one good predictor; humeral calcium with $R^2 = 0.92$. Many other variables showed $p < 10^{-4}$ indicating a non-zero slope, but much scatter was evident. Similarily, scatter was high with femoral phosphate and its best predictor was femoral calcium ($R^2 = 0.87$).

With advancing age of the fetuses, magnesium content of both bones decreased (Exhibits 26-27). The relationship was so slight that the means were not significantly different when grouped into 5 fetal age periods. The only predictor of the magnesium content of femora and humeri was the sodium content of the same bone ($R^2 = 0.30$). This was also the only variable significant at $p < 10^{-4}$. Correlation between femoral and humeral magnesium was 0.51, suggesting either great variations between the two bones or poor method sensitivity.

An inverse, non-linear relationship was seen when sodium content of fetal bones was plotted against developmental age (Exhibits 28-29). As result, in both humeri and femora the sodium content was significantly higher in the bones of 9-10 week old fetuses than the remaining period of 11-20 weeks. Group means were similar but there was less sodium per 100g dry humerus than per 100g dry femur. The best predictor of the sodium content of one bone of a fetus was the sodium content of the corresponding bone of the same fetus ($R^2 = 0.96$) but 7-9 variables showed $p < 10^{-4}$ indicating that the slope of the regression line was not 0.

The Ca/collagen increased in a non-linear fashion when plotted against developmental age (Exhibits 30-31). The Ca/collagen ratio in the femur of 9-12 week old fetuses was significantly lower than that in the 13-20 week period. In the humerus, some overlapping into 3 significant age periods was seen. The ratio had the same start and end value in both bones; humeral Ca/collagen bowed more in the middle range. The best predictor was the Ca/collagen ratio of the corresponding bone at $R^2 = 0.80$, but 6-8 variables clustered below this with $p < 10^{-4}$. These included length, dry weight, and water content of the bone, length of fetus, weight, developmental and gestational age.

A non-linear relationship was seen when the

calcium/phosphate ratio of both bones was plotted against developmental age of fetus (Exhibits (32-33). In fetuses 9-10 weeks old, this ratio in the femur was significantly less than 2.0. In the remaining 13-20 weeks, the ratio was greater than 2.0 in this bone. The Ca/P ratio in the humerus was similar but separated into 3 significant age groups with some overlap. The best predictor of this ratio was the Ca/P ratio in the corresponding bone, although it was seen from the regression data that all other variables except one were significant at $p < 10^{-4}$.

The percent change in weight of each long bone following fat extraction was calculated. If the results expressed in Table VII can be explained by experimental error, no fat was found in the bones of fetuses aged 9-20 weeks.

Table VII. Change in bone weight following fat extraction

Long Bone	(%) Mean	Standard Deviation
femur	-1.5256	2.62
tibia	-0.5943	1.98
fibula	0.4847	3.69
humerus	-0.6093	3.29
radius	-0.4572	3.66
ulna	-0.0900	2.89

B. Maternal Data

Selected variables were coded and compared against each other for simple linear regression data. These potential independent variables were then correlated with the fetal data as dependent variables using stepwise regression analysis. Independent variables which correlated significantly with each other (r = 0.3) were isolated from each other in successive runs.

1. Medical-Growth Information

Means, standard deviations and number of observations for the six variables are shown in Table VIII.

Table VIII. Means and standard deviation of maternal variables

Variable	Sample Size	Unit	Mean	Standard Deviation
Age	76	years	29	8
Height	67	cm	164	6
Weight	66	kg	57.6	8.2
Birth Weight	45	kg	2.9	0.8
Parity	75	no. child.	1.8	2.0
Gravidity	75	no. pregn.	3.3	2.3

A significant relationship was seen between maternal weight and height (R^2= 0.24, p = 0.0001), whereas no relationship was detected between maternal birth weight and present weight (p = 0.6253). Parity and gravidity

correlated significantly with maternal age; the older woman had a greater chance of having more children and more pregnancies than a younger woman. A highly significant relationship was found between parity and gravidity for obvious reasons. Scattergrams for the above are presented in Appendix 2 (Exhibits 34-37). No other relationships between all combinations of the above variables were detected.

When these potential independent variables were analysed in stepwise regression, certain significant correlations were seen. Results are presented in Table IX. First developmental age was held constant; then gestational age was chosen as the significant independent variable.

The data suggest that younger women produced fetuses with longer, more ossified bones at each age of development, with a larger head circumference and a higher Ca/P ratio in the humeri. None of the above relationships were seen when gestational age was held constant.

Maternal weight appeared to be inversely correlated with biochemical indices of the fetal humerus. This suggests that lighter women produced fetuses with more phosphorus, magnesium, calcium and a higher Ca/collagen ratio in the humeri than fetuses of the same developmental age from heavier women. When gestational age was held constant the same relationship held only for humerus phosphorus and magnesium. Interpretation of this finding is difficult;

Table IX. Effect of maternal variables on fetal data

Independ. Var.	Developmental Age constant			Gestational Age constant		
	Depend. Var.	F prob.	Rel.	Depend. Var.	F prob.	Rel.
Age	F-Len	0.0000	-			
	H-Len	0.0000	-			
	HeadC	0.0152	-			
	H-Ca/P	0.0177	-			
	F-oss	0.0276	-			
	H-Oss	0.0415	-			
Weight	H-Pho	0.0007	-	H-Pho	0.0013	-
	H-Mag	0.0079	-	H-Mag	0.0116	-
	H-Cal	0.129	-			
	H-Ca/C	0.0311	-			
Height				H-Pho	0.0381	-
Birth Weight	F-Len	0.0000	-	H-Col	0.0085	+
	H-Len	0.0000	-	HeadC	0.0134	+
	H-Ca/C	0.0054	-	DeAge	0.0210	+
	FootL	0.0071	-			
	F-Oss	0.0142	-			
	GeAge	0.0159	-			
	H-Dry	0.0244	-			
	H-Col	0.0238	+			
Parity	F-Len	0.0000	-	H-Ca/P	0.0058	-
	H-Len	0.0000	-			
	H-Ca/P	0.0009	-			
	FootL	0.0098	-			
Gravidity	F-Len	0.0000	-	H-Ca/P	0.0135	-
	H-Len	0.0000	-			
	H-Ca/P	0.0000	-			
	HeadC	0.0072	-			
	FootL	0.0338	-			
	F-Oss	0.0401	-			
	Weight	0.0445	-			
Socio-econ Score	Weight	0.0116	+			
Socio-econ Group	Weight	0.0066	+	Weight	0.0356	+
Fetal Sex	HeadC	0.0020	-	H-Pho	0.0207	+
				Weight	0.0422	+

F = femur H = humerus

femora and humeri data were strongly correlated with each other yet maternal weight only affected humeral variables. Also, maternal height, which correlated with maternal weight, showed no significant correlation with any of the fetal variables.

Maternal birth weight was inversely correlated with certain physical fetal data. For example, when developmental age was held constant, mothers who weighed more at birth appeared to produce fetuses with shorter bones, shorter feet, less ossified femora and lighter humeri. However there appeared to be a direct correlation between maternal birth weight and humeri collagen. Certain direct relationships were discovered when gestational age was held constant.

There is the suggestion that mothers with fewer children produced fetuses which had longer feet and longer bones than women with a larger family, if developmental age was held constant. There appeared to be little relationship between parity and these dependent variables when expressed as a function of gestational age.

Similar responses were found when number of pregnancies was considered. In addition, when developmental age was kept constant, as gravidity increased, fetal head circumference, weight and femoral ossification decreased. As with parity, no conclusive results were seen when

gravidity was expressed as a function of gestational age.

2. Socio-economic Status

Mean socio-economic score out of a possible 72 was 33.4 $\pm$ 12.9; mean group was 2.7 $\pm$ 0.74. There were 52 observations in each case as the remaining 24 women were unwilling to supply the information. Relative frequencies of the socio-economic groups is shown in Figure 4. The scattergram of socio-economic score versus socio-economic group (Exhibit 38) is presented in Appendix 2. As seen in Table IX, socio-economic data were positively correlated with fetal weight, whether expressed as a score or group, as a function of developmental or gestational age.

3. Sex of Fetus

Results of stepwise regression analysis suggest that females of the same developmental age had a greater head circumference than males, whereas when gestational age was considered, females had lower humeral phosphate values and weighed less than males.

4. Nutritional Data

Relative frequencies, means and standard deviations for each nutrition variable are presented in Figure 5. Sample size was 70 since 6 women were discharged from the

Figure 4. Histogram of Socio-economic Groups

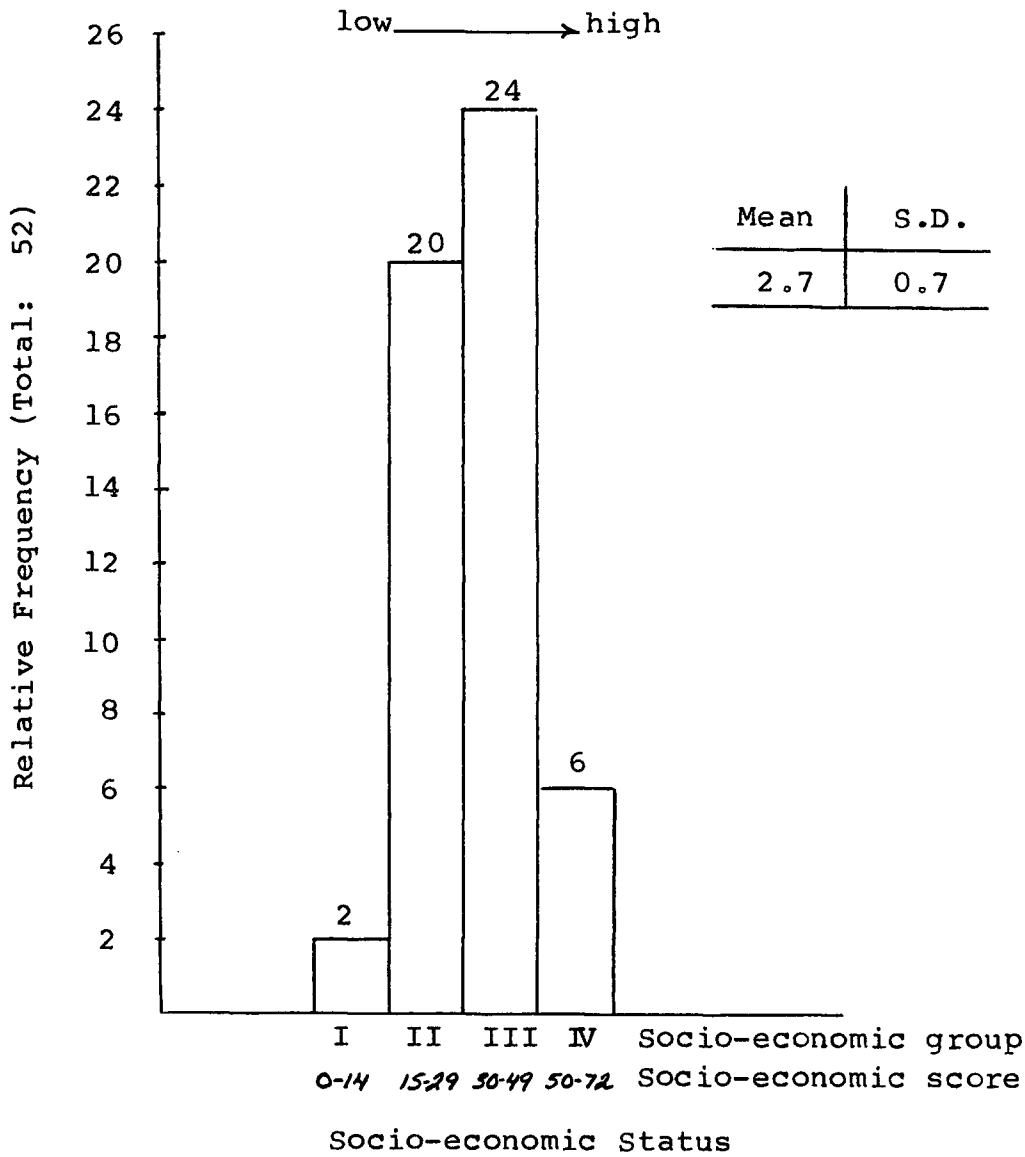


Figure 5a. Histograms of general maternal nutrition score

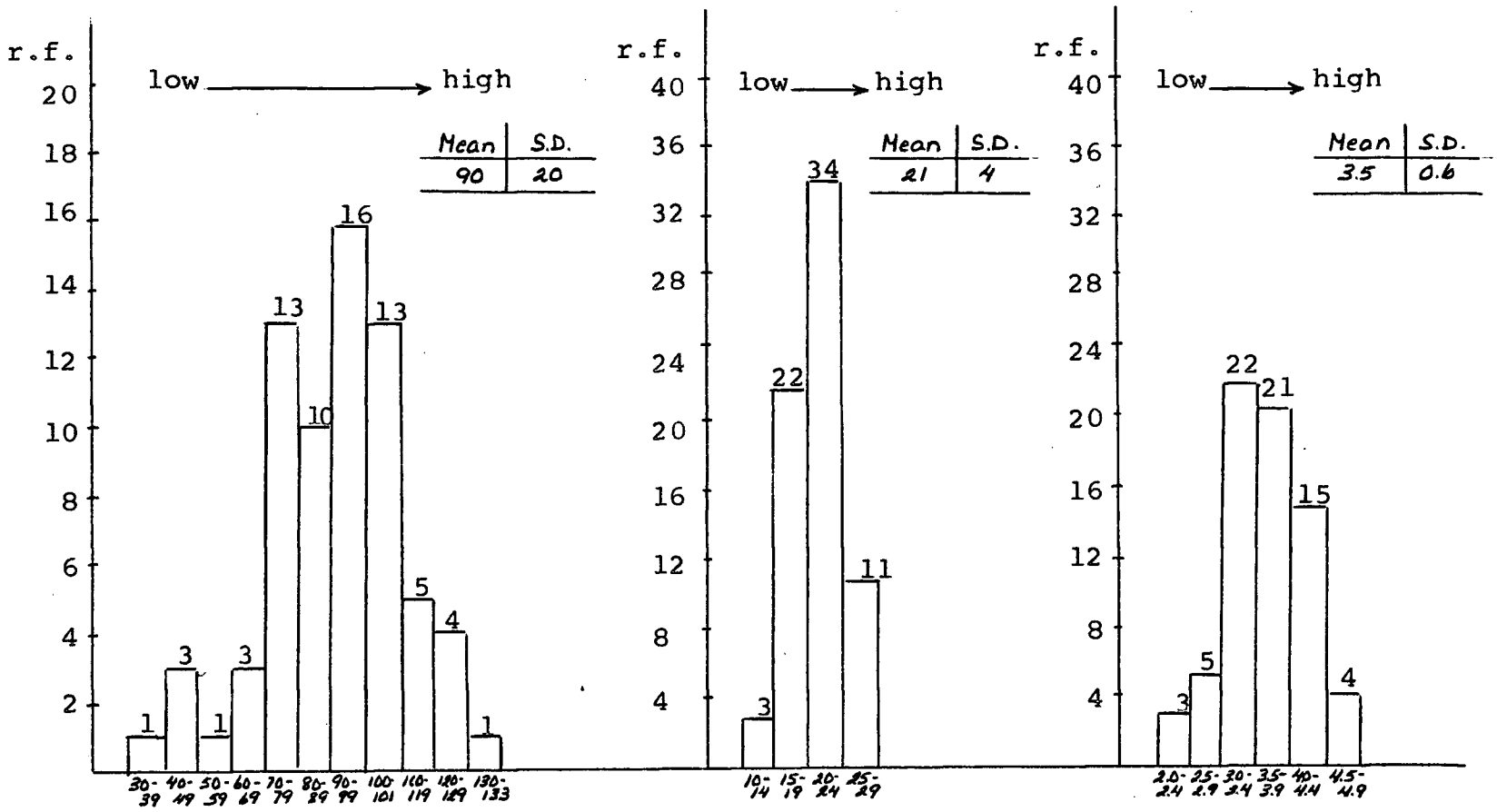
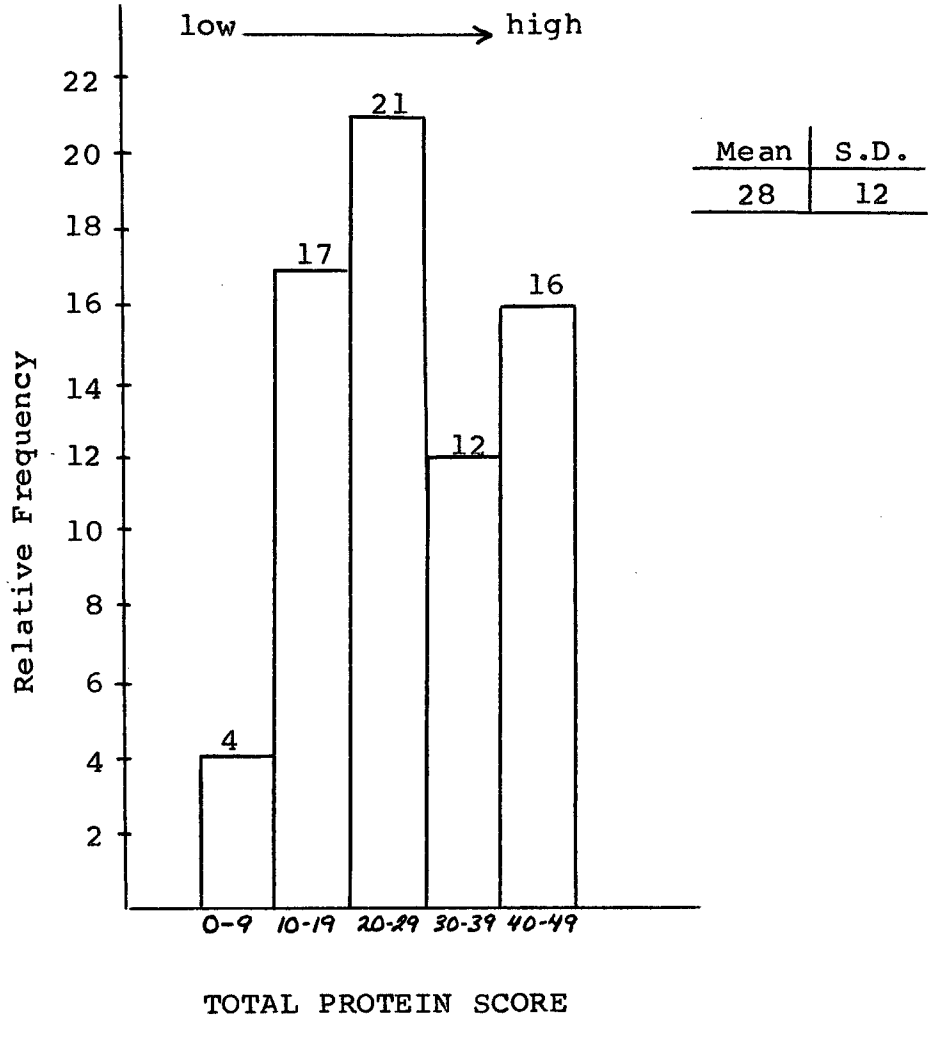


Figure 5b. Histogram of maternal protein score



Mean	S.D.
28	12

hospital before the dietary history could be taken. All scores were arranged on a continuum from low to high and grouped arbitrarily. A normal distribution was obtained for total nutrition score, weighted score and nutrition index; probably only 4 women could be described as having an inadequate diet according to the criteria used. Protein scores clumped at the upper range of the distribution; again only a small number could be classified as having a low animal protein intake.

Total nutrition, weighted score and index were manipulations of the same data and as such correlated well with each other ($R^2 = 0.85$). A direct relationship was also seen between protein score and total nutrition ($R^2 = 0.44$), weighted score ($R^2 = 0.32$) and index ($R^2 = 0.33$). In all of the above correlations, F probability was significant at $p < 10^{-4}$. Scattergrams are included in the Appendix (Exhibits 39-44). No further relationships were detected between nutritional, maternal or socio-economic data at the 5% level of significance.

Table X presents the significant relationships that resulted when nutritional factors were tested as potential independent variables in multiple regression analysis.

Protein score of the maternal diet did not affect any fetal variables, either when developmental or gesta-

Table X. Effect of nutritional variables of fetal data

Independent Variable	Developmental Age constant			Gestational Age constant		
	Depend. Var.	F prob.	Rel.	Depend. Var.	F prob.	Rel.
Total nutrition	F-Len	0.0000	+	H-Dry	0.0165	+
	H-Len	0.0000	+	F-Dry	0.0199	+
	H-Dry	0.0001	+	F-Pho	0.0317	+
	F-Dry	0.0015	+	F-Len	0.0404	+
	F-Pho	0.0365	+	H-Len	0.0435	+
Weighted score	F-Len	0.0000	+	H-Dry	0.0010	+
	H-Len	0.0000	+	F-Dry	0.0012	+
	F-Dry	0.0000	+	F-Len	0.0087	+
	H-Dry	0.0000	+	H-Len	0.0117	+
	F-Pho	0.0496	+	F-Pho	0.0262	+
Nutrition index	F-Len	0.0000	+	H-Dry	0.0010	+
	H-Len	0.0000	+	F-Dry	0.0012	+
	F-Dry	0.0000	+	F-Len	0.0079	+
	H-Dry	0.0000	+	H-Len	0.0105	+
				F-Pho	0.0284	+
Protein score	no significant rel.			no significant rel.		

F = femur, H = humerus

tional ages were held constant. Total nutrition, weighted score and index were comparable in effect, with total nutrition score predicting the greatest number of variables with the lowest probability. Results suggest that general nutrition of the mother was directly related to the length and dry weight of both long bones studied. Although this relationship held when gestational age was considered, probability of chance relationship was greater and more scatter was seen.

D I S C U S S I O N

It can be shown that there are drawbacks to basing the age of the fetus on its crown-rump length. Aside from experimental error in measurement fetuses of the same length are not necessarily the same age from conception, and vice versa. Just as the estimated gestational age of a newborn is an important clinical datum which must not be disregarded whatever the infant's birth weight or length, estimation of fetal age from maternal dates must be considered. Large scatter in regression data from gestational age against all other variables can be explained in three ways: (a) fetuses the same age in utero grow at highly variable rates; (b) for psychiatric reasons, irregular menstruation or poor memory, the mother was unable to give an accurate date of her last menstrual period; (c) if conception did not occur 14 days after the start of her last menstrual period, estimated gestational age would be inaccurate. Any or all of these reasons could contribute to errors in the gestational age assigned to each specimen. Battaglia (219) has suggested that reliable menstrual histories be selected. Since such selection could eliminate 10-40% of the sample, does this remainder constitute a normal reference group (220)? A number of researchers

ignore this situation by proposing a variety of other specimens as models for the study of fetal biology, e.g. rhesus monkey. Because normal fetuses of exactly known gestational age are rarely available for analysis, human studies like the present one must be content with expressing results according to developmental age and thus fetal length.

With increasing developmental age of the fetuses studied, the length, dry weight and extent of ossification increased in both the humerus and femur. These factors showed a strong positive correlation with each other. The weights, rather than the lengths of the limb bones were found to result in a more reliable estimate of fetal weight. Trotter, in his research on older fetuses (81), described a significant correlation between weights of the total osseous skeleton to birth weight and to lengths of the osseous diaphyses of the humerus and femur; each increased with age.

The many tables in the literature (61-68) describing the developmental sequence of both membraneous and endochondral ossification have been concerned with the time of appearance not the extent or length of ossified diaphyses. The present work has shown conclusively that long bone ossification as detected from silver radiography is a simple and accurate parameter of fetal age. This could be substantiated by performing the technique on a large backlog

of therapeutically aborted fetuses. The resulting bone age table, based on the lengths of the ossified diaphyses of femora and humeri, could then be used to date spontaneously aborted specimens.

Bone composition results are in agreement with Dickerson's research (86) on the human femur although the present study was concerned with both a younger, more narrow age range, and a larger total sample size. The fundamental change in the composition of a bone during development is an increase in its degree of ossification. This is accompanied by a decrease in the percentage of water (85). Hammett's observation was substantiated in this study. It was found that bone length and ossification best predicted water content. However, because cleaning of bone for analysis takes considerable time and controlled conditions to determine accurately the percentage of water, it is customary to express composition of bone tissue on a dry fat-free basis.

The results of this study seem to confirm Dickerson's statement (86) that no fat is present in the fetal femur during the 12-28 week age range. However, the effectiveness of petroleum ether to penetrate the bone and to break the lipoprotein complexes in the marrow could be questioned. A micro-soxhlet apparatus would have been a more sensitive technique although problems in drying and weighing a bone of such size (1-400mg) would still have to be solved.

Collagen did not increase significantly in the femurs of fetuses 9-20 weeks developmental age. Although similar results were found by Dickerson (86) in femoral cortical bone, it is surprising that collagen would not increase when expressed as g/100g whole bone. Femoral collagen was the only variable examined that did not predict developmental age at the 5% level of significance. Humeral collagen was significantly higher in 11-20 week fetuses than in 9 - 10 week specimens. Differences in humeral and femoral collagen content are not readily explainable.

Whereas calcium increased linearly in both femora and humeri of 9-20 week old fetuses, inorganic phosphorus increased in a non-linear manner. Therefore the Ca/P ratio (indicator of bone mineralization) was significantly less than 2.0 at 9-10 weeks, and constant at 2.0 in the remaining age range. Dickerson (86) and Swanson (94, 95) reported relatively constant Ca/P ratios when expressed per 100g dry fat-free solids. However, fetuses less than 12 weeks were not studied by either researcher. The results of the present work suggest that either the ratio of calcium to inorganic phosphate deposition is not constant with bone growth, or younger specimens have proportionately larger amounts of organic phosphorus resulting in contamination. Perhaps the ratio increase after 10 weeks was due to a decrease in the proportion of phosphate from ester phos-

phates - a large part of the phosphorus in the bones of immature fetuses being present in ester form.

Because crystals of bone mineral are principally laid down in association with collagen fibrils, the Ca/collagen ratio gives a measure of the degree of saturation of collagen fibrils. The ratio was found by Dickerson (86) to change very little during growth in humans. This is in agreement with the currently accepted view that collagen fibrils are rapidly mineralized to about 80% saturation soon after they are laid down. The results of this study indicate a significantly lower ratio in younger fetuses. Again, bone composition of 9-10 week old fetuses has not been reported. Perhaps the calcification mechanism is not fully developed to mineralize a surplus of collagen fibrils in the cartilaginous model. This explanation is reasonable because calcification of long bones does not begin until the fetus is 8 weeks old (57).

Sodium content of fetal bones, 9-10 weeks old was significantly higher than in bones from fetuses aged 11-20 weeks. Sodium is found in the bone in the extra cellular fluid, in the hydrated layer of bone crystals and in the bone crystals themselves. Although a dynamic process is occuring, the trend seen here confirms the findings of Swanson and Iob (94, 95).

Magnesium is also thought to be inversely related to fetal age (94, 95). The constant value of bone magnesium found in this study, and the poor correlation between humeral and femoral magnesium would suggest poor method sensitivity.

Some comments can be made concerning the fetal model evolved in this project. Generally, physical variables best predicted other physical variables. Similarly, biochemical variables best predicted other biochemical variables. With the exception of magnesium and collagen, which remained constant, all biochemical variables correlated significantly with physical data at better than the 5% level. This is a reasonable, but until now undocumented, finding.

In this study, femoral and humeral data were found to be comparable. Until now, studies of skeletal growth and development in the human fetus have been limited to the femur. Data presented herein show that the rate of growth between the humerus and the femur, as assessed by physical and biochemical variables, is similar. Coefficients of Determination associated with femur variables were generally higher than those associated with corresponding humerus variables when plotted against developmental age (less scatter about the regression line). However, humerus results generally had a more significant F probability than did femur results, and therefore a greater separation into

age groups was seen in this bone. The reason for this pattern is not readily explainable.

The study did not detect a consistent sex difference among the fetal variables analysed. Roche (84) and others have observed that ossification is more advanced in the female than in the male during the last three months prenatal and at birth, whereas the birth weights of males are generally higher. Perhaps these effects are not detectable until after 20 weeks developmental age.

Caution must be exercised in drawing conclusions from the effect of most maternal variables on fetal variables. A statistically significant correlation does not establish a causal relationship. A direct correlation found between maternal variables (e.g. age, parity, gravidity) and newborn variables (e.g. birth weight and length) has been observed (32, 48, 49). Apparently this relationship extends into earlier fetal life. Previous studies have shown that maternal height, weight and birth weight (33-38) are directly correlated with certain newborn growth parameters. The negative correlation found in this study contradicts previous findings. The reason for this contradiction is unclear. It is not inconceivable that maternal age, weight, birth weight, parity and gravidity could be correlated in some way with fetal skeletal growth, but factors such as ethnic origin (23, 24), maternal anxiety (27, 28), smoking

(39, 40), season (143) and paternal variables must also be considered in the analysis. One of these additional variables could be mediating the observed effect of socio-economic status on fetal weight (49-51).

The potential for undertaking studies on the mothers of the experimental fetuses was rather limited because of the emotional factors associated with the performance of a therapeutic abortion. Technical problems associated with conducting research in a hospital manifested themselves through attitudes of nursing personnel and facilities available. Accordingly, it was important to limit the amount of information obtained from the mother without prejudicing the needs of the study.

This limitation curtailed the scope and accuracy of the maternal dietary history. The study concerned a unique group of women. Seventy-three out of seventy-six had been granted their abortion for psychiatric reasons. They were easily upset, very guilt-ridden, and generally reluctant volunteers. It was not feasible to validate the questionnaire with blood or urine samples, or with 7-day dietary histories following discharge. Whereas the questionnaire itself could have been validated on normal volunteers, it would have been of doubtful significance to extrapolate from a normal situation to the abortion patients.

The interview placed emphasis on the quantity and

variety of the diet in a relative sense. Scores were arranged on a continuum from low to high. Crump (45) devised this scale from a study of 483 pregnant women in Nashville, Tennessee. He validated the oral dietary history using 7-day food records from the same patients. Diets were classified as "poor" (scores 24-33), "fair" (34-52), "good" (53-70) and "excellent" (71-133). From his calculations a score of 60 was found to represent an intake of two-thirds the Recommended Daily Allowance during pregnancy. Ninety-five percent (mean plus/minus two standard deviations) of the nutrition scores in the present study fell into Crump's "good" or "excellent" classifications. Only 4 women could be considered to have "fair" diets during pregnancy. This would indicate that scores were taken from the upper range of the normal population distribution; not surprising considering that the procedure and cost of a therapeutic abortion effectively limits the operation to those in the middle-to-upper socio-economic range.

Because the scores represented a continuum from fair to excellent, it was considered valid to regress nutritional data against fetal data. Perhaps egg, cheese, meat and milk content of the maternal diet gave an inaccurate protein score, for this variable showed no relationship with any of the fetal parameters examined. On the other hand, nutrition score (a measure of protein, vitamins, minerals and calories) directly correlated with length and dry weight

of both humeri and femora. In turn, dry weight of bones was the best predictor of fetal weight, and length of bone best predicted bone ossification. Both bone length and weight were significant predictors of fetal length and developmental age, and good indicators of bone calcium, Skeletal growth and maturation are obviously under control of a fundamental biological growth mechanism (1). These data suggest that nutritional factors may affect the efficiency with which this mechanism functions.

In conclusion, normal skeletal growth and development of the human fetus can be described in terms of a correlation between whole fetal measurements, physical growth and biochemical composition of either the femur or the humerus. Using this model, further nutritional research could be conducted to explore the relationship between fetal bone growth and diet during pregnancy. Length and weight of either bone, as well as being significantly correlated with maternal nutrition in this study, are relatively simple and accurate parameters to analyse, and would allow considerable increase in sample size during a similar time period. Bone calcium would be the best predictor of bone composition and could be compared with cord blood calcium and maternal blood calcium. Biochemical findings could then be related to maternal consumption of milk and other calcium-rich foods during pregnancy. Possibilities are as numerous as the number of dimensions

presented; study in this area offers an interdisciplinary blend of nutrition, embryology, biochemistry and psychology.

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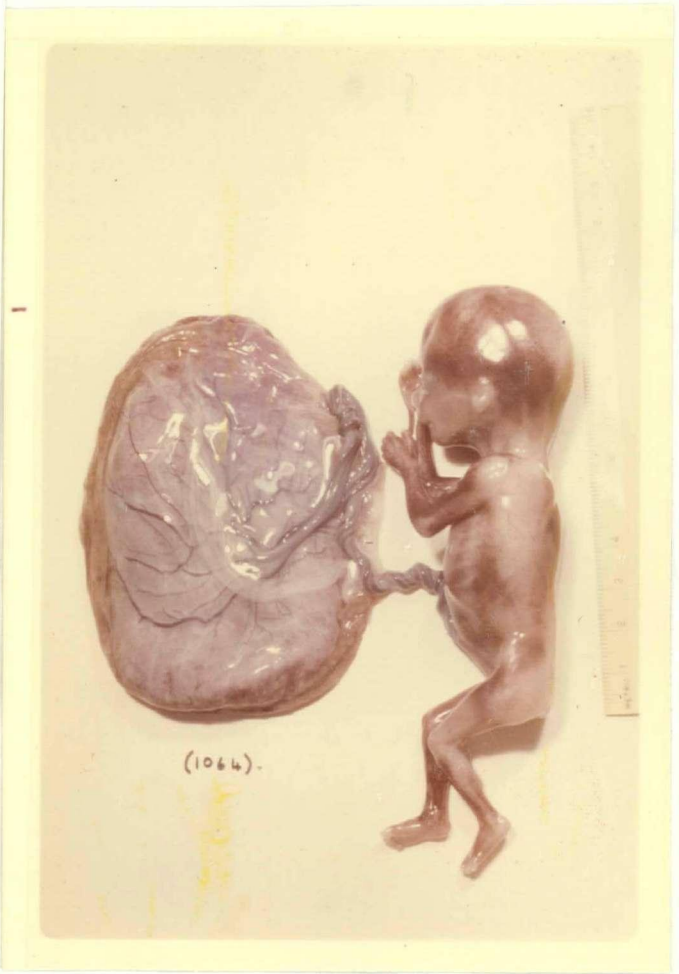
APPENDIX 1

Table XI. Crown-rump length versus developmental age of fetus

CR length (mm)	days after ovul'n	CR length (mm)	days after ovul'n	CR length (mm)	days after ovul'n
1.0	18	40-41	58	113-114	97
1.5	20	42	59	115-116	98
1.8	22	43-44	60	117-118	99
2.0	22	45-46	61	119-120	100
2.8	24	47-48	62	121-122	101
3.0	25	49	63	123-124	102
3.5	26	50-51	64	125-126	103
4.0	27	52-53	65	127-128	104
4.5	28	54	66	129	105
5.0	28	55-56	67	130-131	106
6.0	29	57-58	68	132-134	107
6.5	29	59-60	69	135-136	108
7.0	30	61-62	70	137-138	109
7.5	31.5	63-64	71	139	110
8.0	32	65	72	140-141	111
9.5	33	66-67	73	142-143	112
10.0	33	68-69	74	144-145	113
11.0	34	70-71	75	146-147	114
12.0	35	72-73	76	148-149	115
13.0	35	74	77	150	116
14.0	36	75	78	151-152	117
15.0	37	76-78	79	153	118
16.0	37	79-80	80	154-155	119
17.0	38	81	81	156-157	120
18.5	39	82	82	158	121
20.0	40	83-86	83	159-160	121
21.0	41	87-89	84	161-162	122
22.0	41	90-91	85	163-164	124
23.0	43	92-93	86	165	125
24.0	43	94	87	166	128
25.0	44	95-97	88	167	130
26.0	45	98-99	89	168	131
27.0	51	100-101	90	169	132
28-29	52	102-103	91	170-172	133
30-31	53	104	92	173-174	134
32-34	54	105-106	93	175-177	135
35-36	55	107-108	94	178-179	136
37	56	109-110	95	180	137
38-39	57	111-112	96		

Plate 1. Specimen in intact sac



2. Placenta and fetus
Female: Crown-rump length - 114mm
Developmental age - 97 days

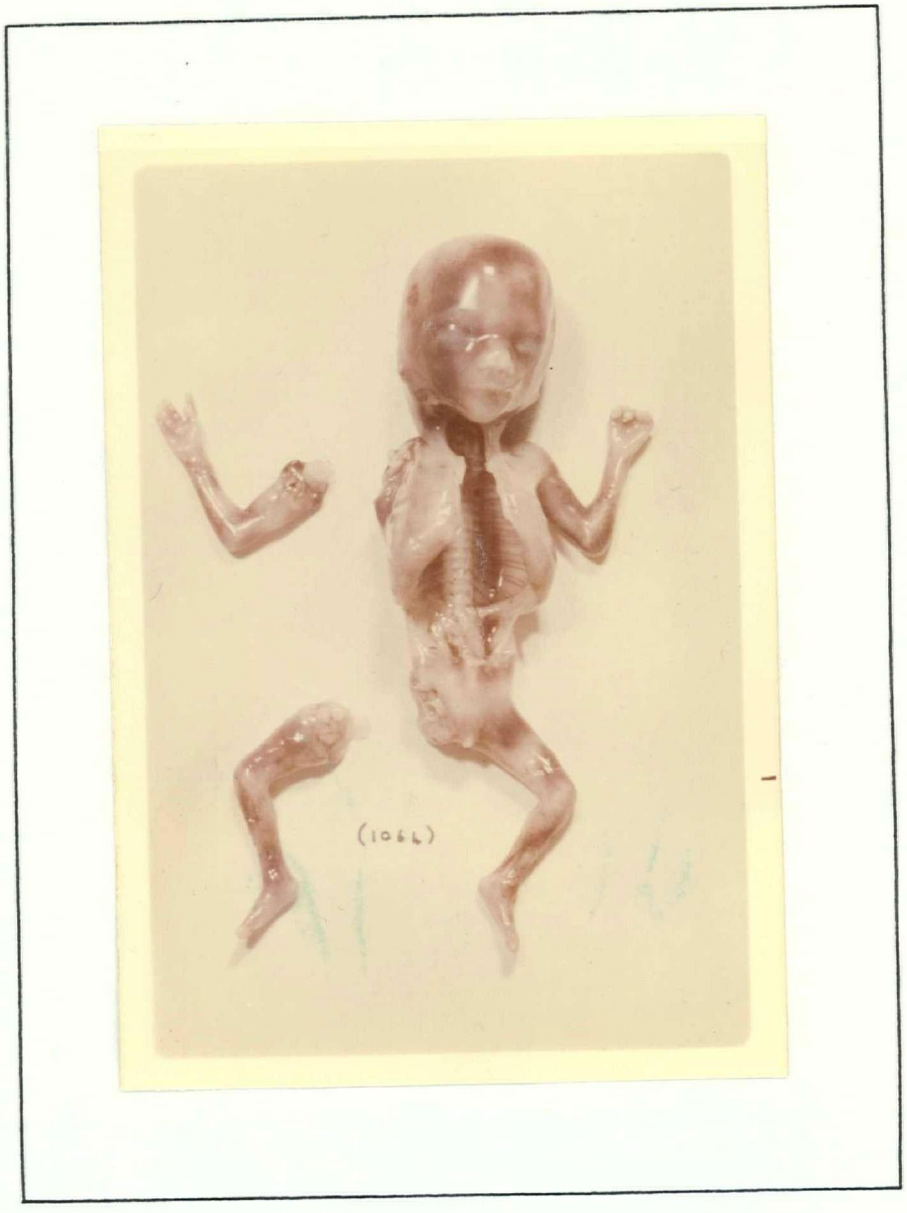


Plate 3. Eviscerated fetus with right arm and leg removed

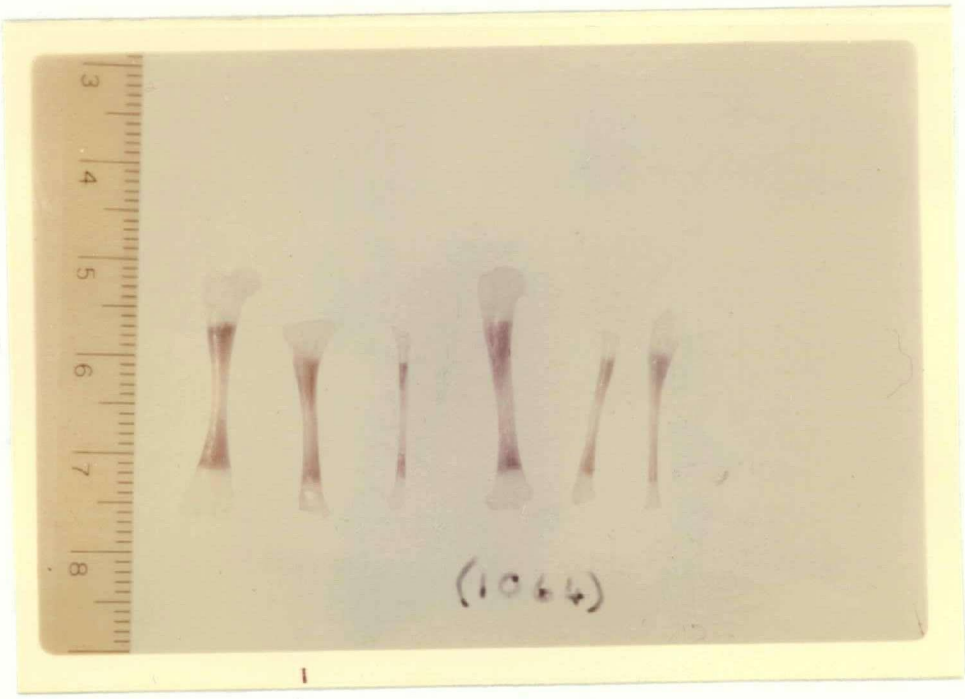


Plate 4. Six fetal long bones: cleaned
(femur, tibia, fibula, humerus, radius, ulna)

Plate 5. Six fetal long bones; dried
(femur, tibia, fibula, humerus, radius, ulna)

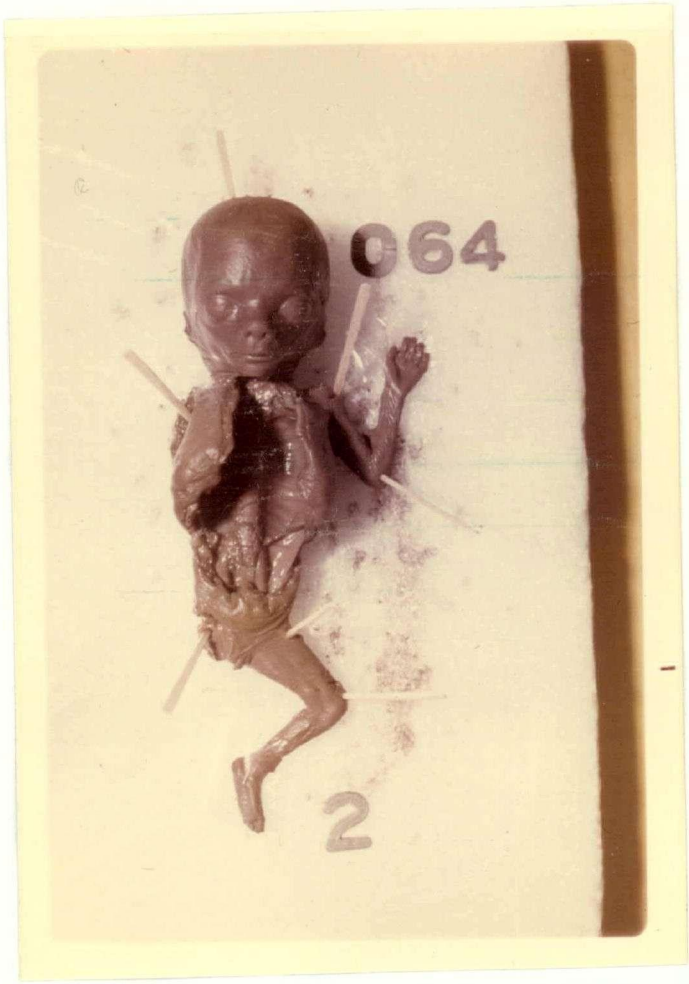


Plate 6. Fetus prepared for radiography after silver nitrate treatment

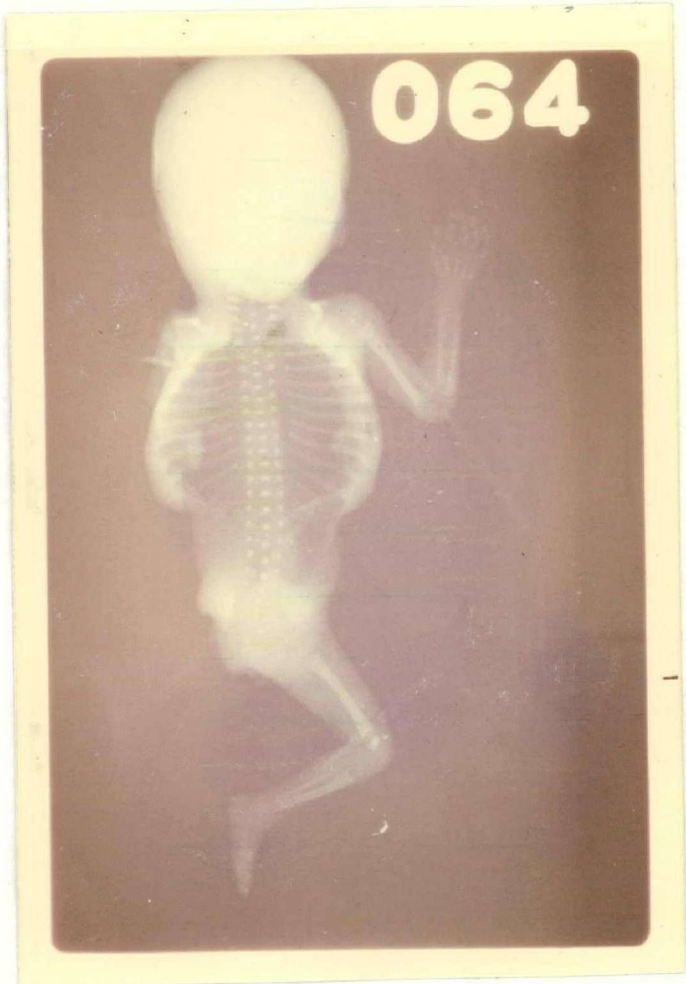


Plate 7. Radiograph; fetus in formalin for 1 day

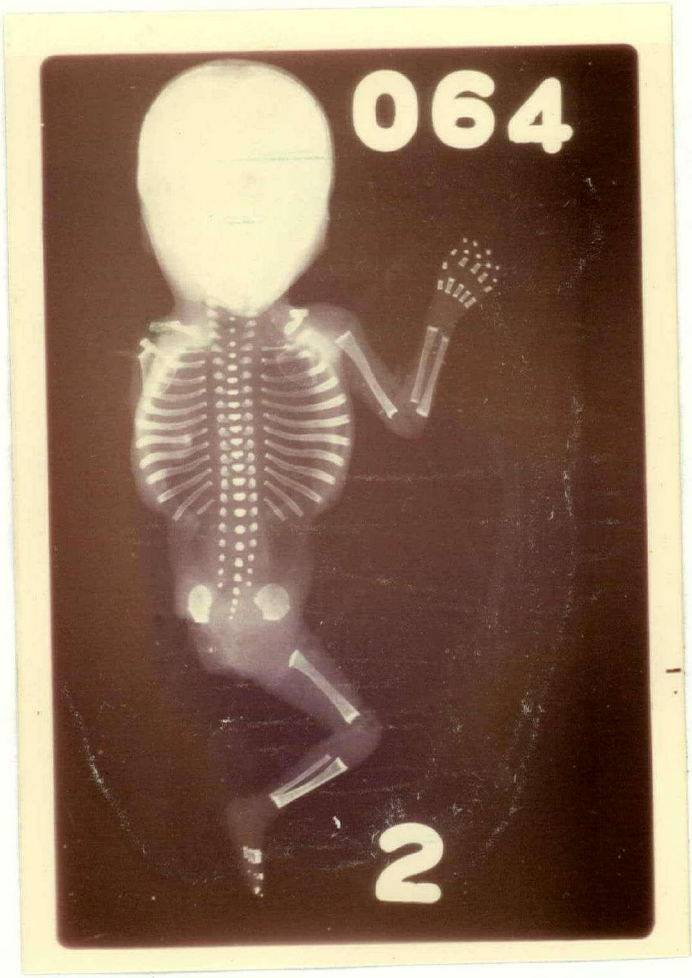


Plate 8. Radiograph; fetus in silver nitrate for 6 days

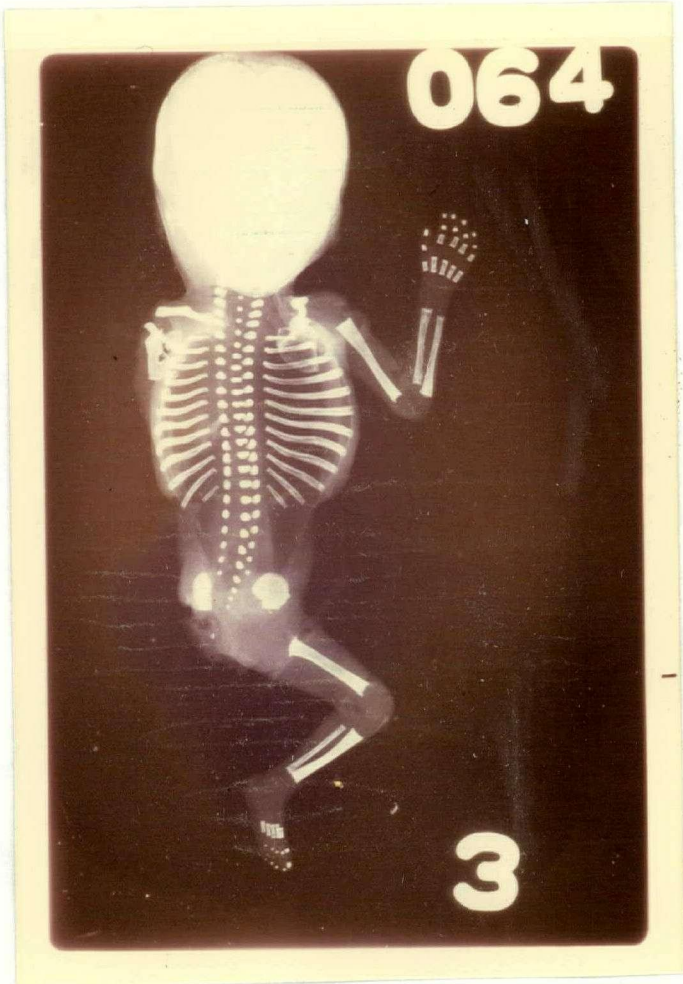


Plate 9. Radiograph; fetus in silver nitrate for 10 days

Table XII. Minimum formalin treatment for silver radiography

Crown-rump length mm	Formalin Treatment days
40- 60	2
60- 75	3
75- 90	4
90-110	5
110-130	6
over 130	7

Table XIII. Optimum silver nitrate treatment for fetal radiography

Crown-rump length mm	Silver nitrate treatment days
40- 60	2
60- 70	3
70- 80	4
80- 90	5
90-100	6
100-110	7
110-120	8
120-130	9
130-140	10
over 140	11

Table XIV. Exposure time for fetal radiographs on G. E. Model F unit (58kv., 10ma)

Crown-rump length mm	Exposure time sec
40- 60	1
60- 90	2
90-130	3
130 and over	4

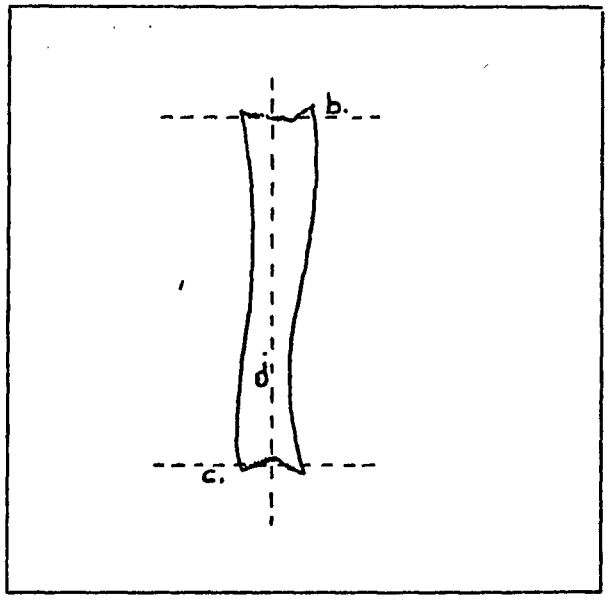


Figure 6. Measurement of ossified shaft of fetal bone

a. Length of ossification of long bone (femur, tibia, fibula, humerus, radius, ulna)

b. Width of ossification at proximal metaphysis of each long bone

c. Width of ossification at distal metaphysis of each long bone

If necessary, a bone was divided into two or three parts by pencil lines perpendicular to the plane of the bone; length of each section was measured and total length found by addition.

Measurements were recorded in millimeters.

Form 1. Dietary History

Specimen Number 1037

A. Daily pattern recall:

time	food	amount
Breakfast	juice or fruit	
	cereal	
	toast	
	tea	
Mid-Morning		
Lunch	soup	
	sandwich or salad	
	fruit	
	tea	
Mid-Afternoon		
	juice or fruit	
Dinner	meat	
	1 vegetable	
	1 potato	
	dessert (fruit, pie, cake)	
	tea	
Mid-Evening		
	bun, crumpet, toast	

B. Food Frequency Questionnaire

How often and how much do you eat of the following types of foods?

Milk never (as a drink)

Cheese cheddar, cottage - 4 servings/week

Eggs 2/week as such (plus usual cooking)

Meat mainly beef, pork

no liver

Fish 3 servings/month

Poultry 1 serving/week

} 10 servings per week

Bread > 4 slices or equivalent/day

Cereal products prepared mostly with milk

6 servings/week

Vegetables: green peas, beans, broccoli, salads

> 2 serv/day yellow carrots, corn, cauliflower

potatoes daily

Fruit: citrus oranges, grapefruit

> 2 serv/day non-citrus apples, bananas, berries

Sweets very rarely

Fats butter on bread, buns > 2T/day

Beverages tea, juices

Nutritional Supplements Ca pills during Nov, irregular use since

Cigarettes does not smoke during pregnancies (about 1 pkg/week otherwise)

C. Likes and Dislikes

Dislikes: milk
liver
beets, cooked celery
Craved peanuts during pregnancy

D. General Comments

Little difference between pregnant and non-pregnant eating patterns.
Patient takes calcium pills when she feels "pain in her joints."

Form 2. Nutritional Status

Specimen Number 1037

Dietary Intake by Specific Food Groups (Crump et al. Am. J. Obstet. Gynec. 77:562, 1959)

	Food Group (number servings per week)					
	Milk	Meat Eggs Cheese	Cereal	Veg.	Fruit	Butter
5 Excellent	28+	21+	28+	14+	14+	28+
4 Good	21-27	16-20	21-27	11-13	11-13	21-27
3 Fair	14-20	11-15	14-20	8-10	8-10	14-20
2 Poor	7-13	6-10	7-13	5- 7	5- 7	7-13
1 Very Poor	3- 6	3- 5	3- 6	3- 4	3- 4	3- 6

Food group	no. servings per week	rating	
Milk	0	1	16 Protein Score (49)
Meat, eggs, cheese	16	4	
Cereal	>28	5	
Vegetables	>14	5	
Fruit	>14	5	
Butter	>14	5	
	86 Total Nutrition Score (133)	25 Weighted Nutrition Score (30)	4.2 Nutrition Index (5)

Comments:

Probably adequate in most nutrients.
Ca and Vitamin D intake?

Form 3. Socio-economic Status

Specimen Number 1037

Short Form Socio-economic Index (Crump et al. J. Pediat. 51:678, 1957)

Score	Occupation of Father (or mother)	Education of Mother	Education of Father	Marital Status
9	professional, semi-professional	college 4	college 4	give average of known values for "married" (4 - 8)
8	official, propriet. manager, col. student	college 3	college 3	
7	clerical	college 2	college 2	
6	skilled	college 1	college 1	
5	semi-skilled	grade 12	grade 12	
4	protective service high school student	grade 11	grade 11	divorce, sep. desert., widow (average 1-3)
3	service (except protect or domestic)	grade 10	grade 10	
2	domestic service	grade 9	grade 9	
1	farm labourer	grade 8	grade 8	
0	unskilled labourer	less than 8	less than 8	single

Socio-economic Group	Score X 2	Range
Group I	0 - 14	low ↓ high
Group II	15 - 29	
Group III	30 - 49	
Group IV	50 - 72	

Index	Answer Given	Score
Occupation of Father	accountant	7
Education of Mother	Grade 12	5
Education of Father	University 2	7
Marital Status	married	7
	TOTAL SCORE (X2)	52
	SOCIO-ECONOMIC GROUP	IV

APPENDIX 2

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
DEAGE	CRLEN	36.34	0.5418	0.1185D 05	0.0	0.5446	0.4976D-02	1.283	0.9953

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

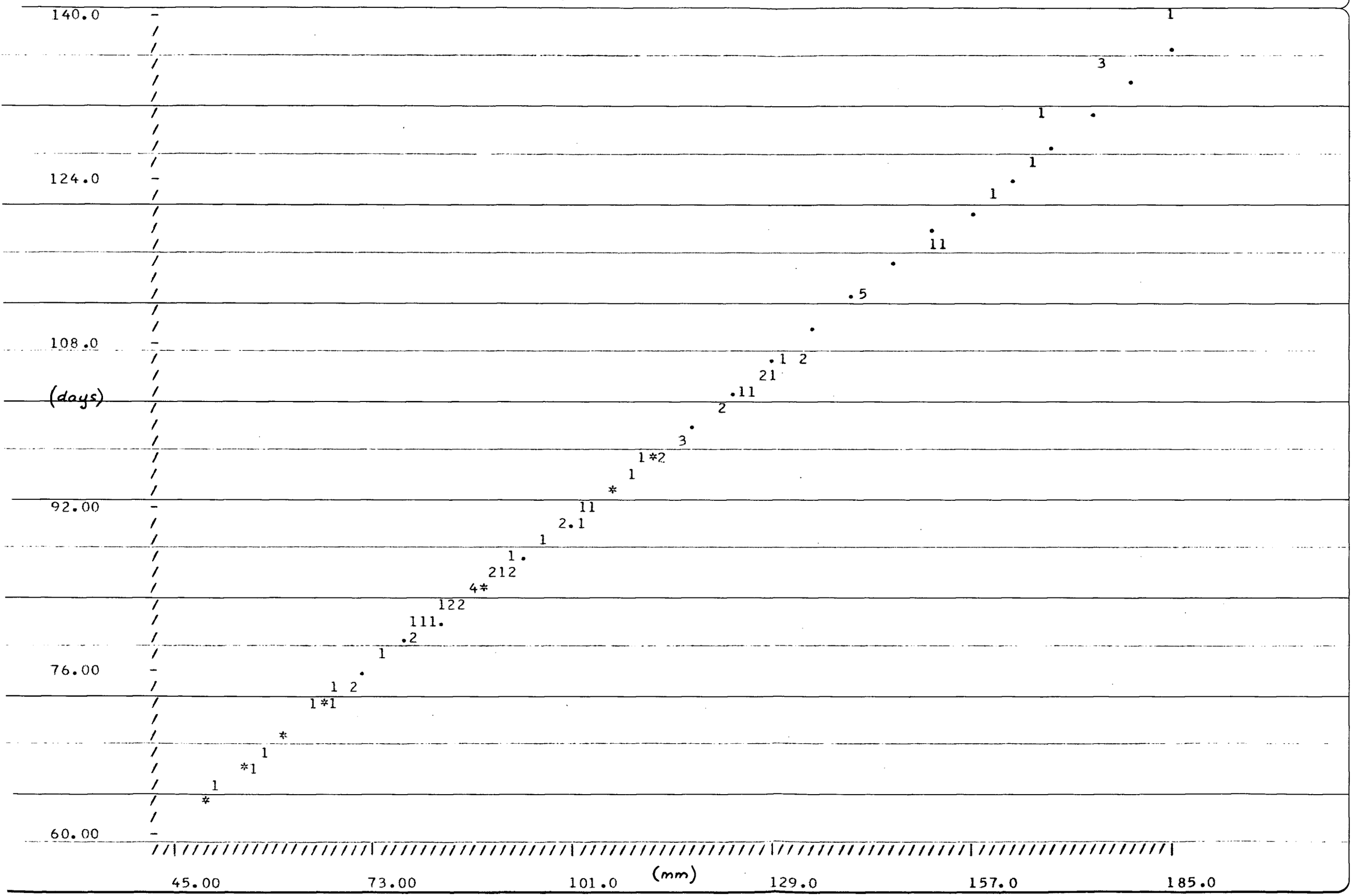


EXHIBIT 1. Developmental age (Y) versus crown-rump length (X)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
HEADC	DEAGE	-72.47	1.919	1355.	0.0	4.927	0.5212D-01	7.299	0.9603

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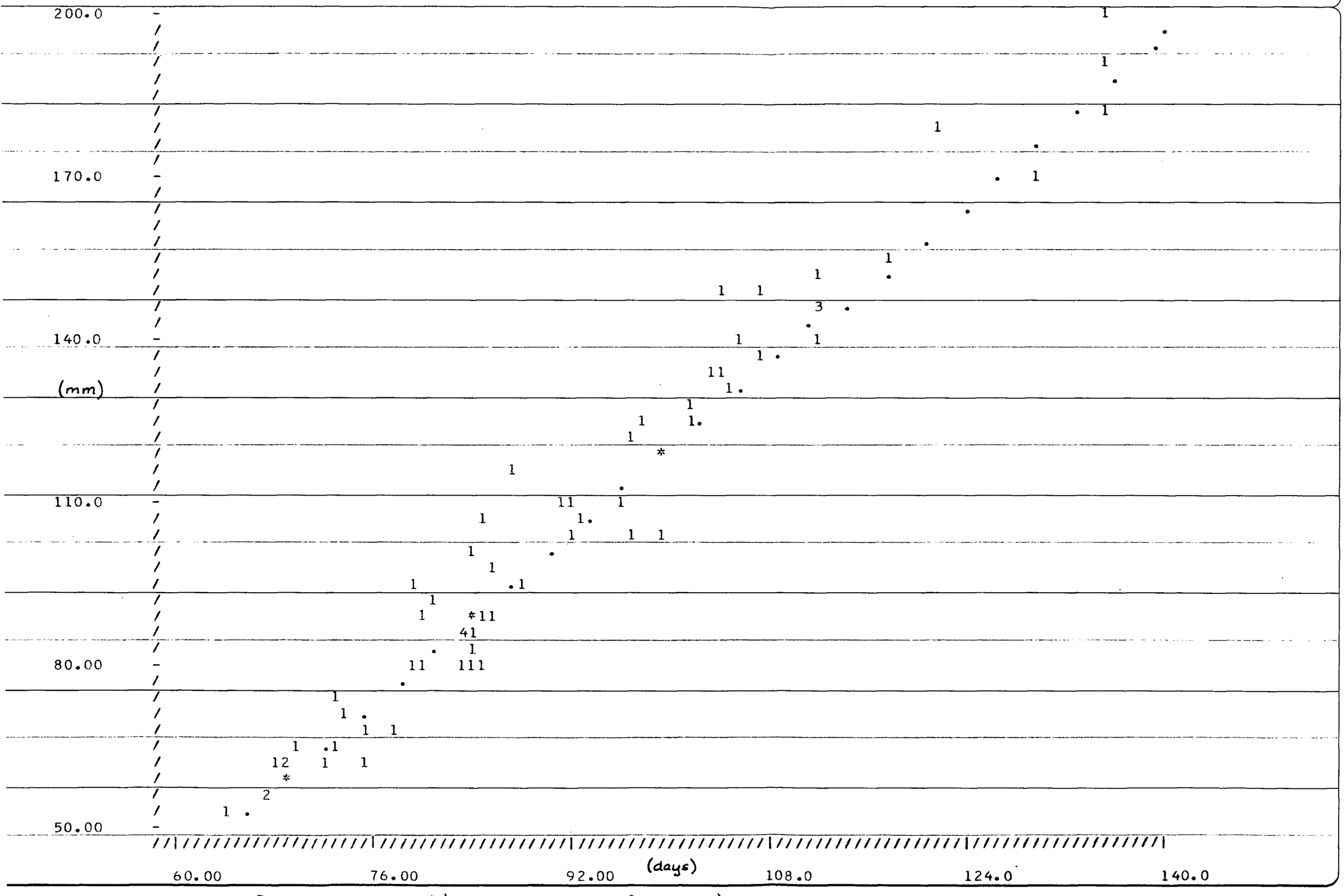

Exhibit 2. Developmental age (X) versus head circumference (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
FOOTL	DEAGE	-20.93	0.4236	1080.	0.0	1.218	0.1289D-01	1.805	0.9507

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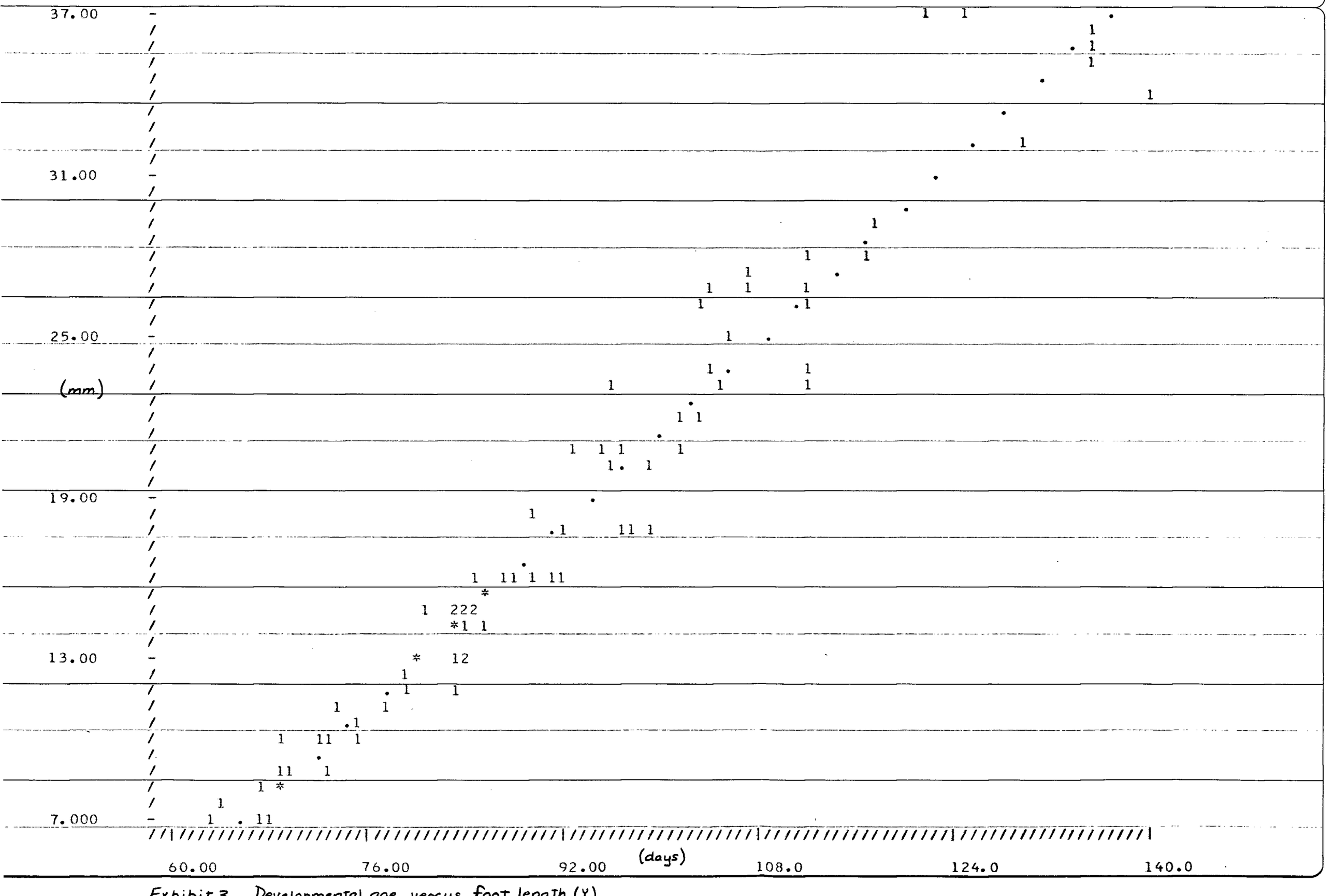

Exhibit 3. Developmental age versus foot length (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
WEIGH	DEAGE	-338.4	4.613	402.6	0.0	21.74	0.2299	32.20	0.8779

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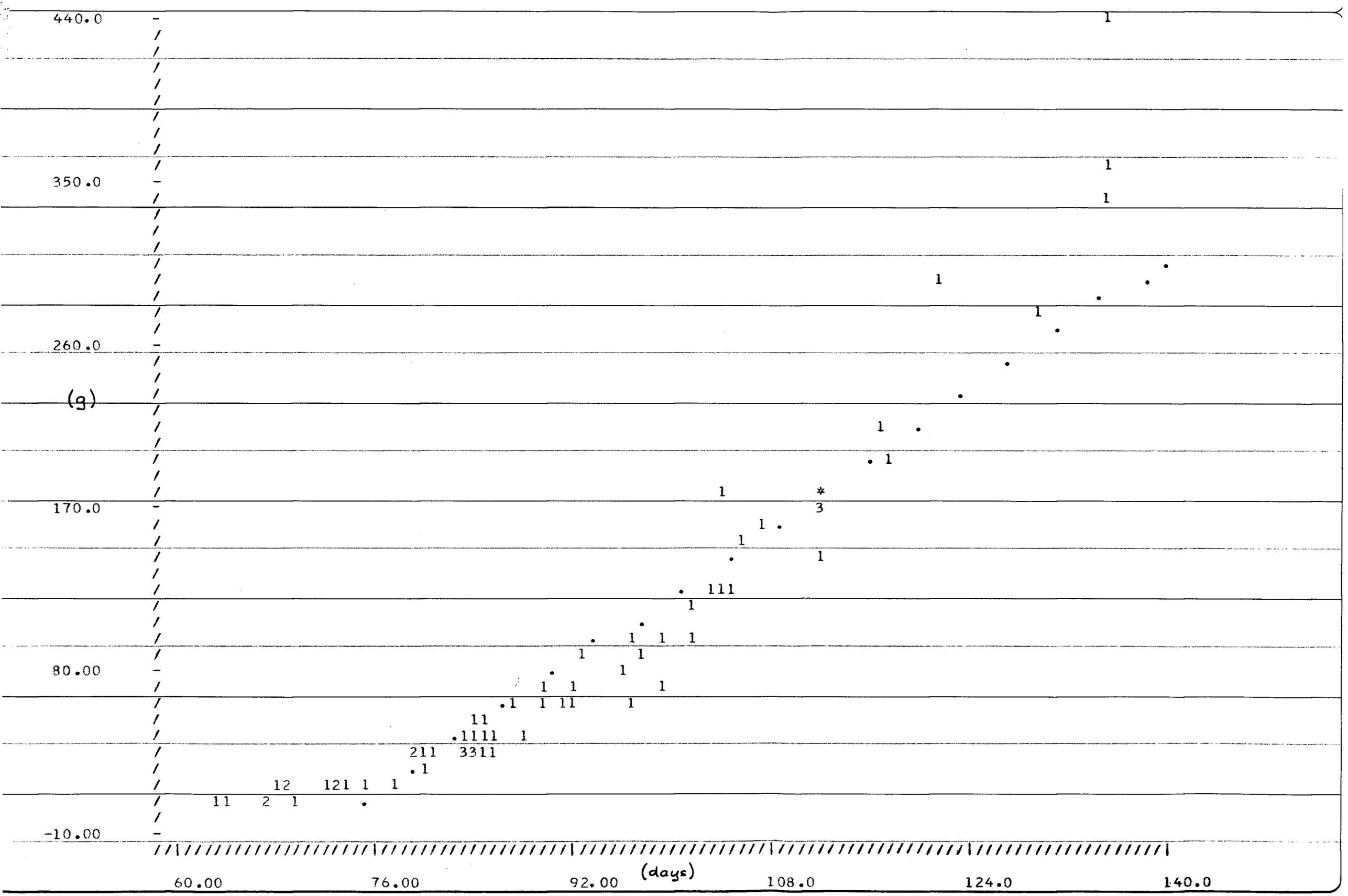

Exhibit 4. Developmental age (x) versus fetal weight (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
DRUWP	DEAGE	-1.278	0.2541	173.0	0.0000	1.826	0.1932D-01	2.706	0.7555

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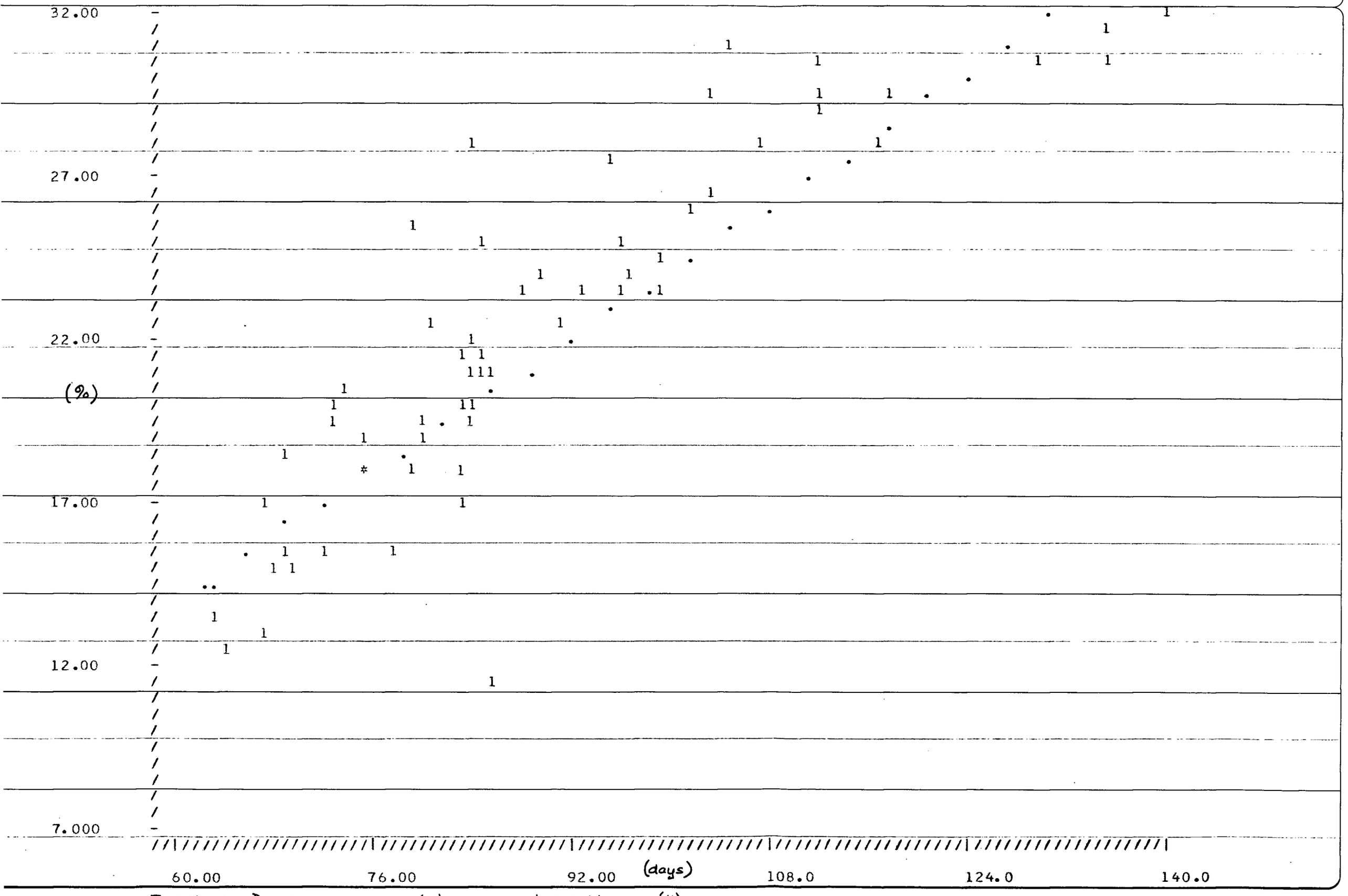

Exhibit 5. Developmental age (X) versus skeletal index (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
GEAGE	DEAGE	1.940	0.9971	114.3	0.0000	8.815	0.9325D-01	13.06	0.6712

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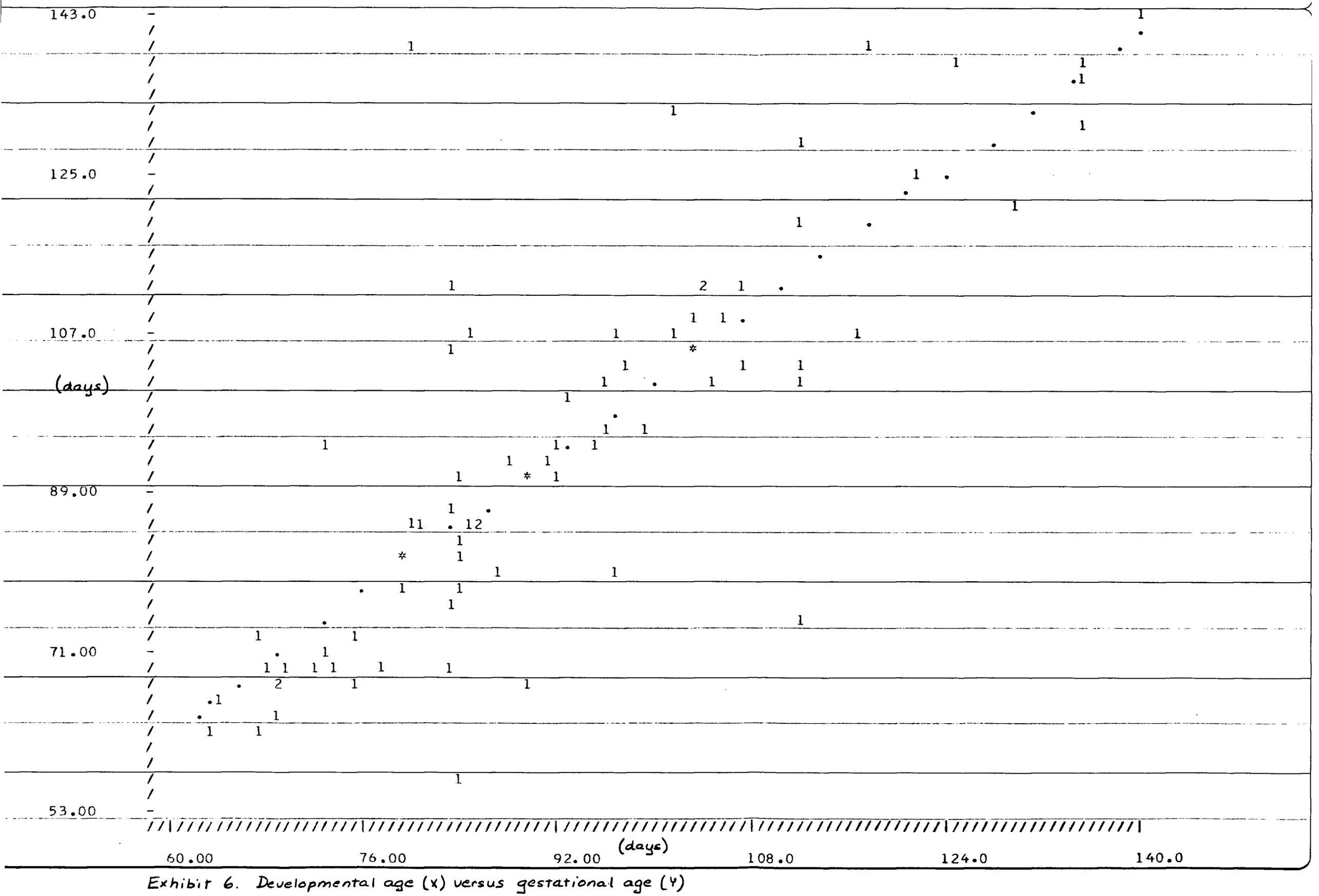

Exhibit 6. Developmental age (x) versus gestational age (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
FOOTL	GEAGE	-9.780	0.2979	128.6	0.0000	2.548	0.2627D-01	4.477	0.6967

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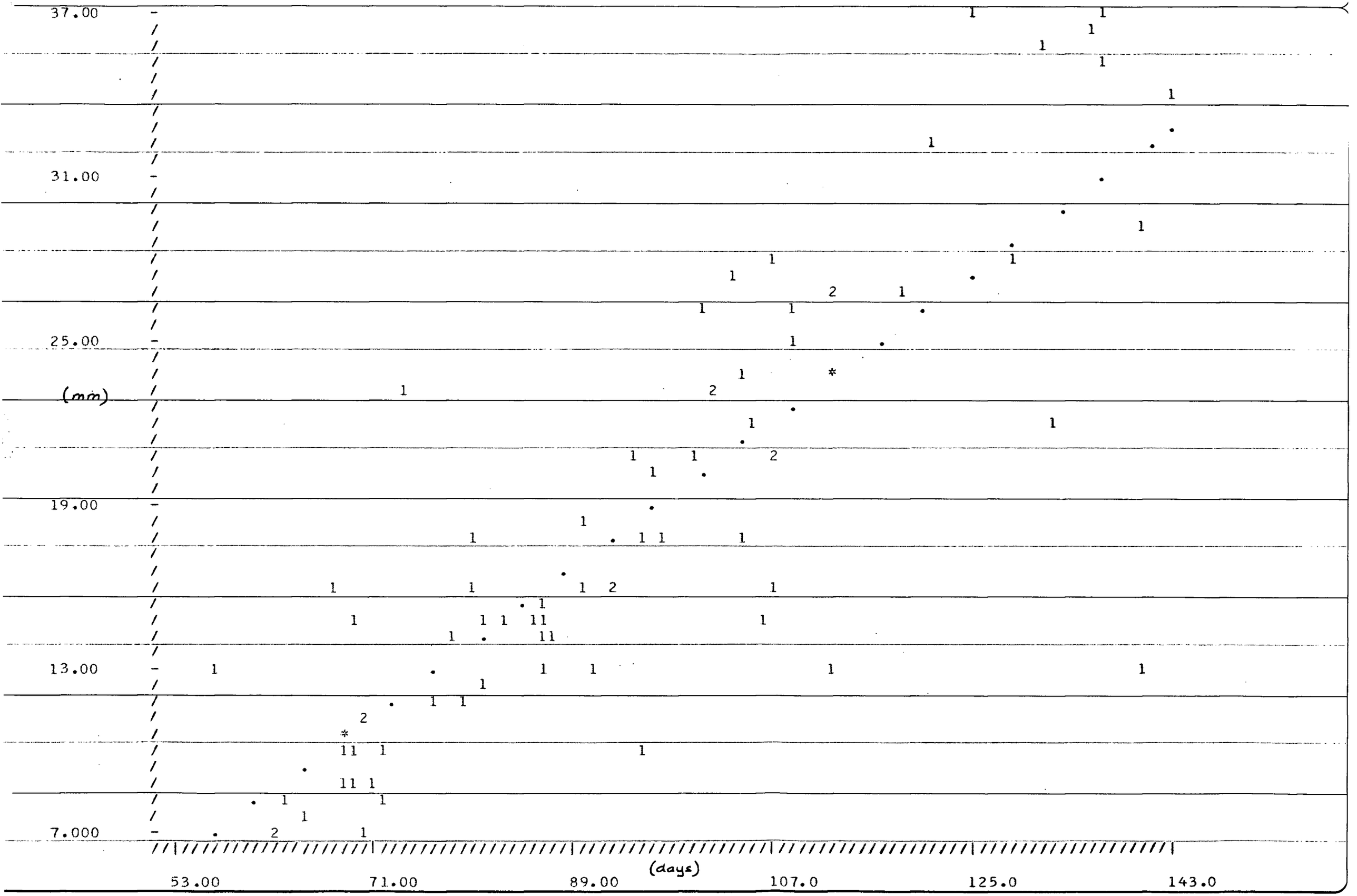

Exhibit 7. Gestational age (X) versus foot length (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
GEAGE	CRLEN	38.04	0.5414	114.3	0.0000	5.544	0.5065D-01	13.06	0.6711

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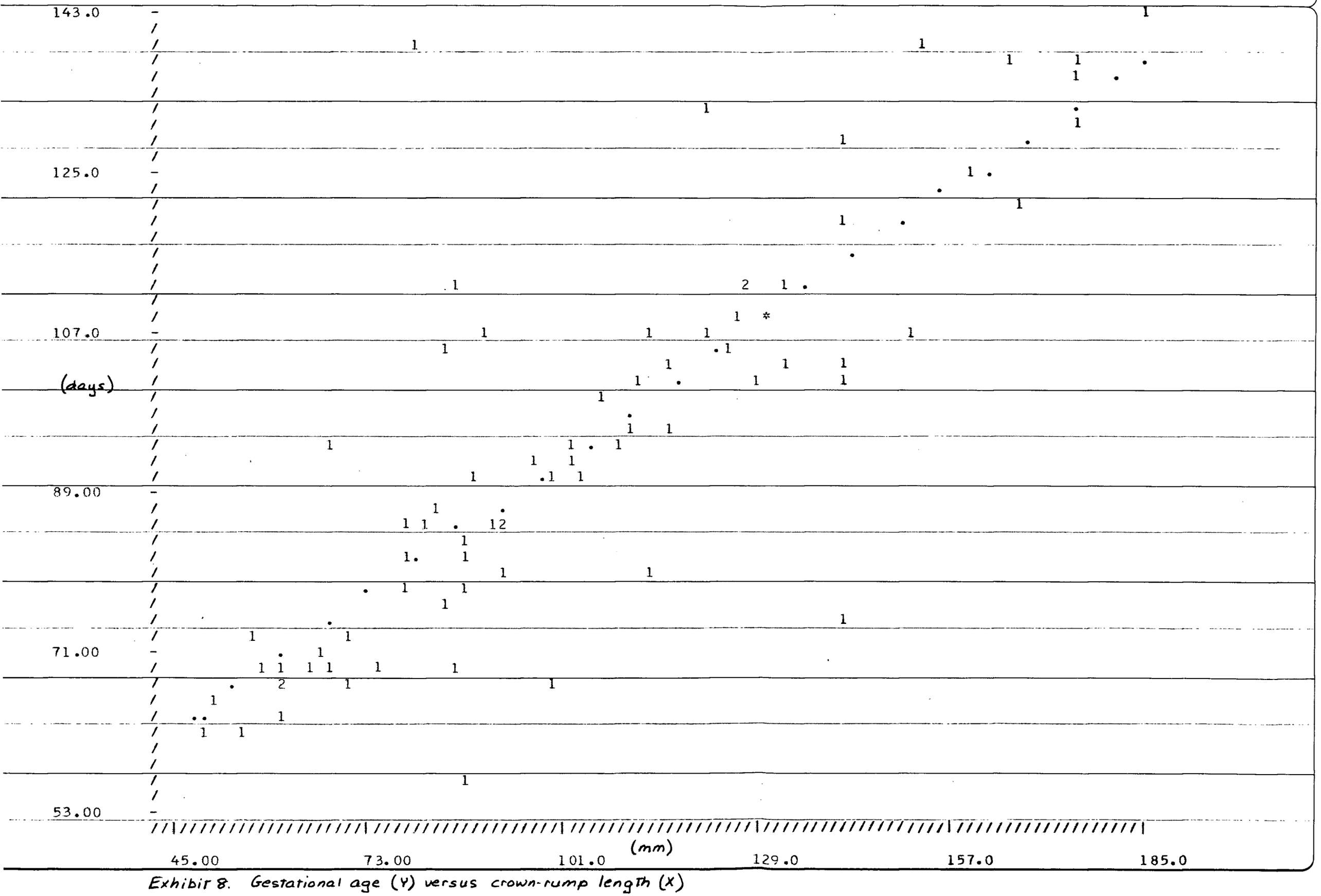

Exhibit 8. Gestational age (Y) versus crown-rump length (X)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
HEADC	GEAGE	-18.48	1.313	111.7	0.0000	12.05	0.1243	21.17	0.6660

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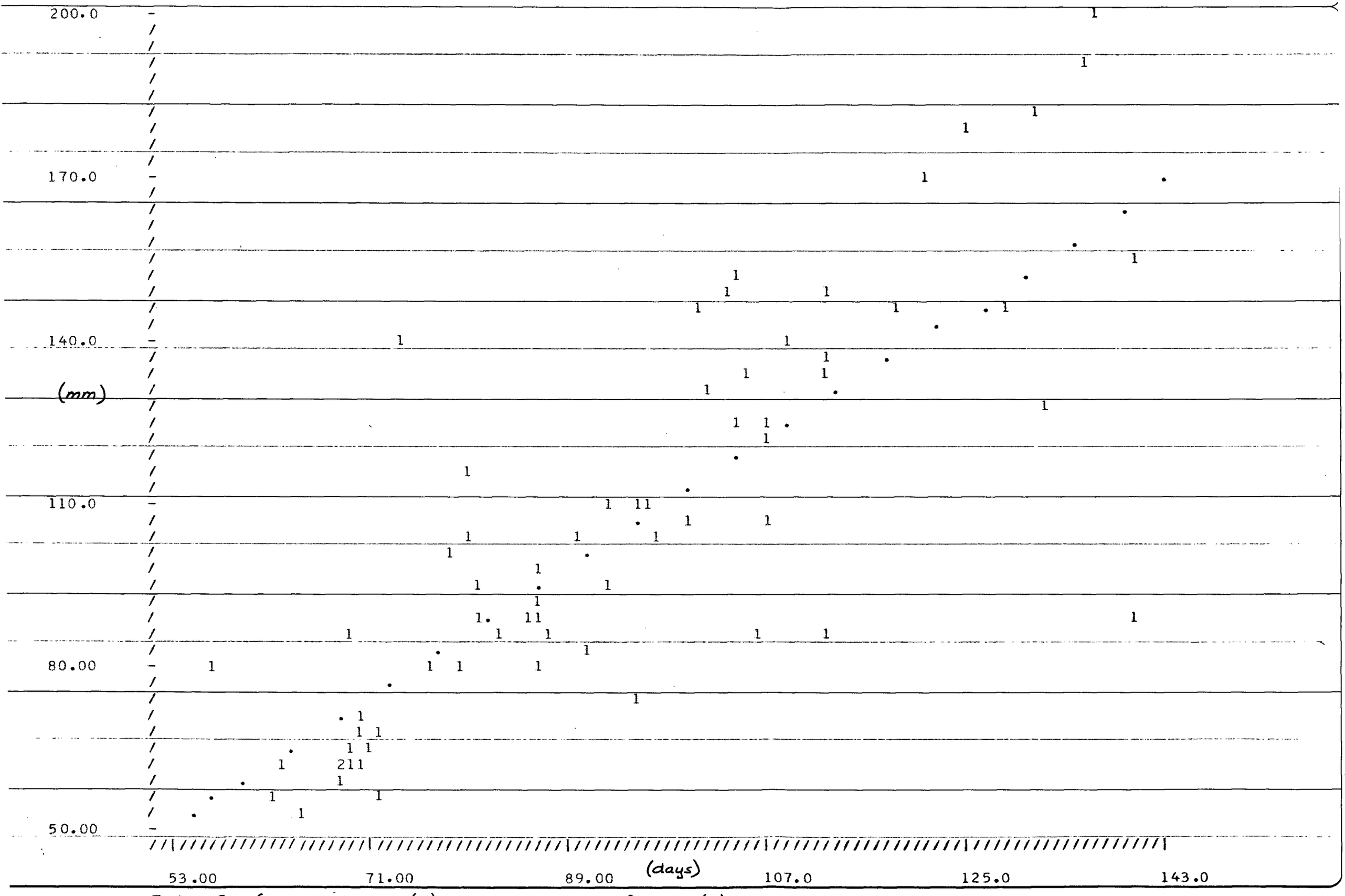

Exhibit 9. Gestational age (X) versus head circumference (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
WEIGH	GEAGE	-200.7	3.074	76.44	0.0000	34.10	0.3516	59.91	0.5772

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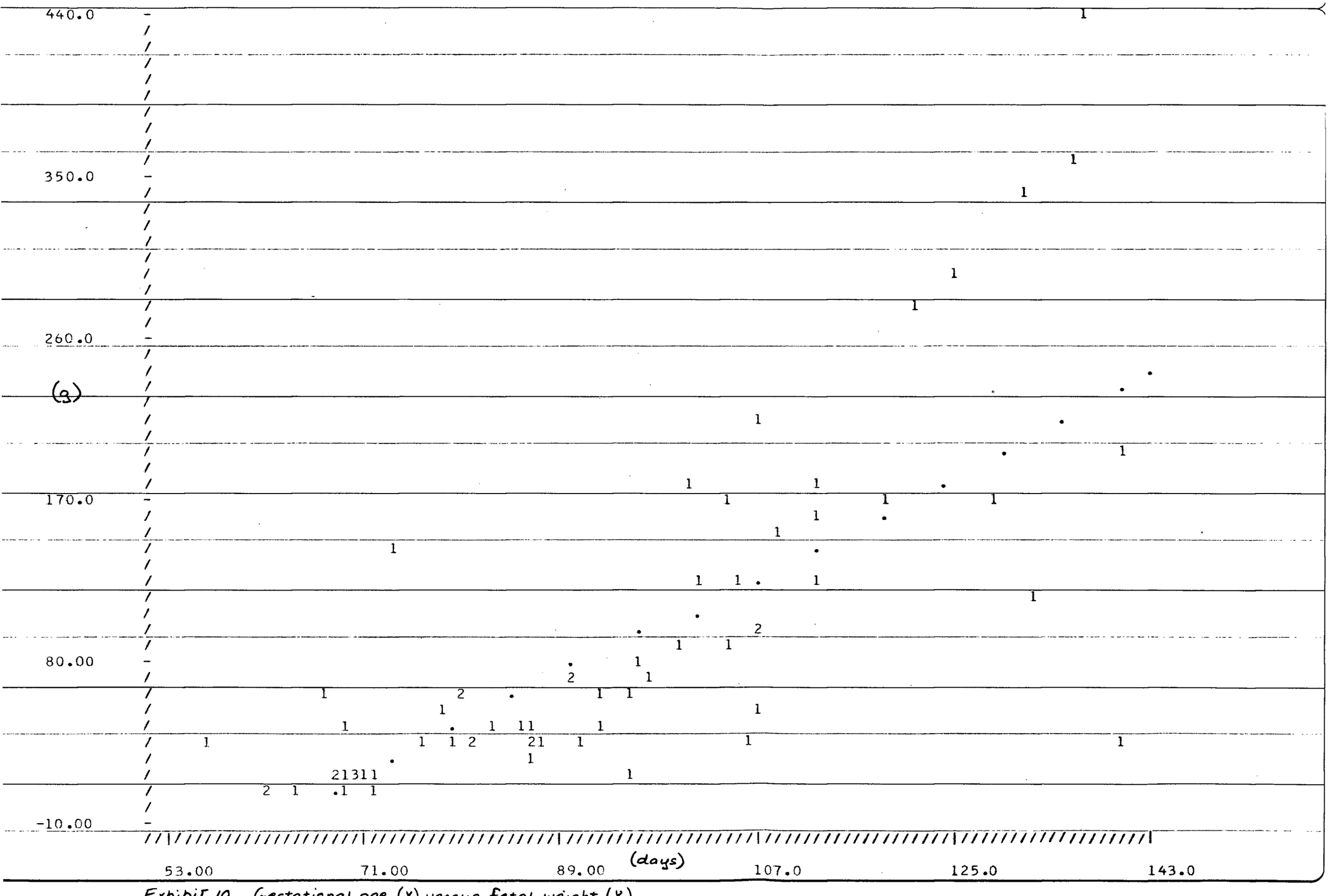

Exhibit 10. Gestational age (X) versus fetal weight (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
DRUWP	GEAGE	6.439	0.1679	53.45	0.0000	2.228	0.2296D-01	3.913	0.4884

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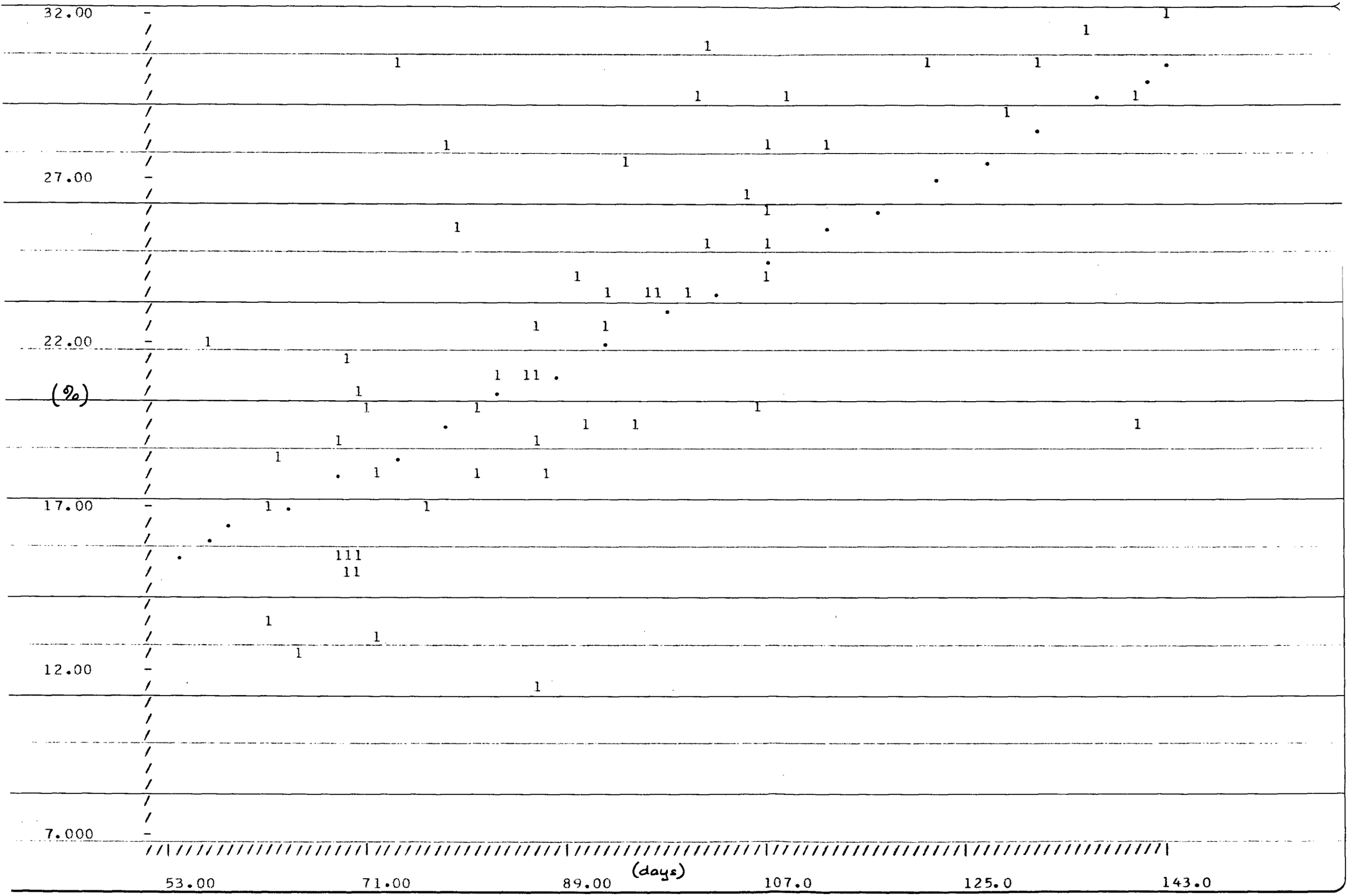

Exhibit 11. Gestational age (x) versus skeletal index (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
F-DRY	DEAGE	-412.2	5.191	319.9	0.0	27.44	0.2902	40.64	0.8510

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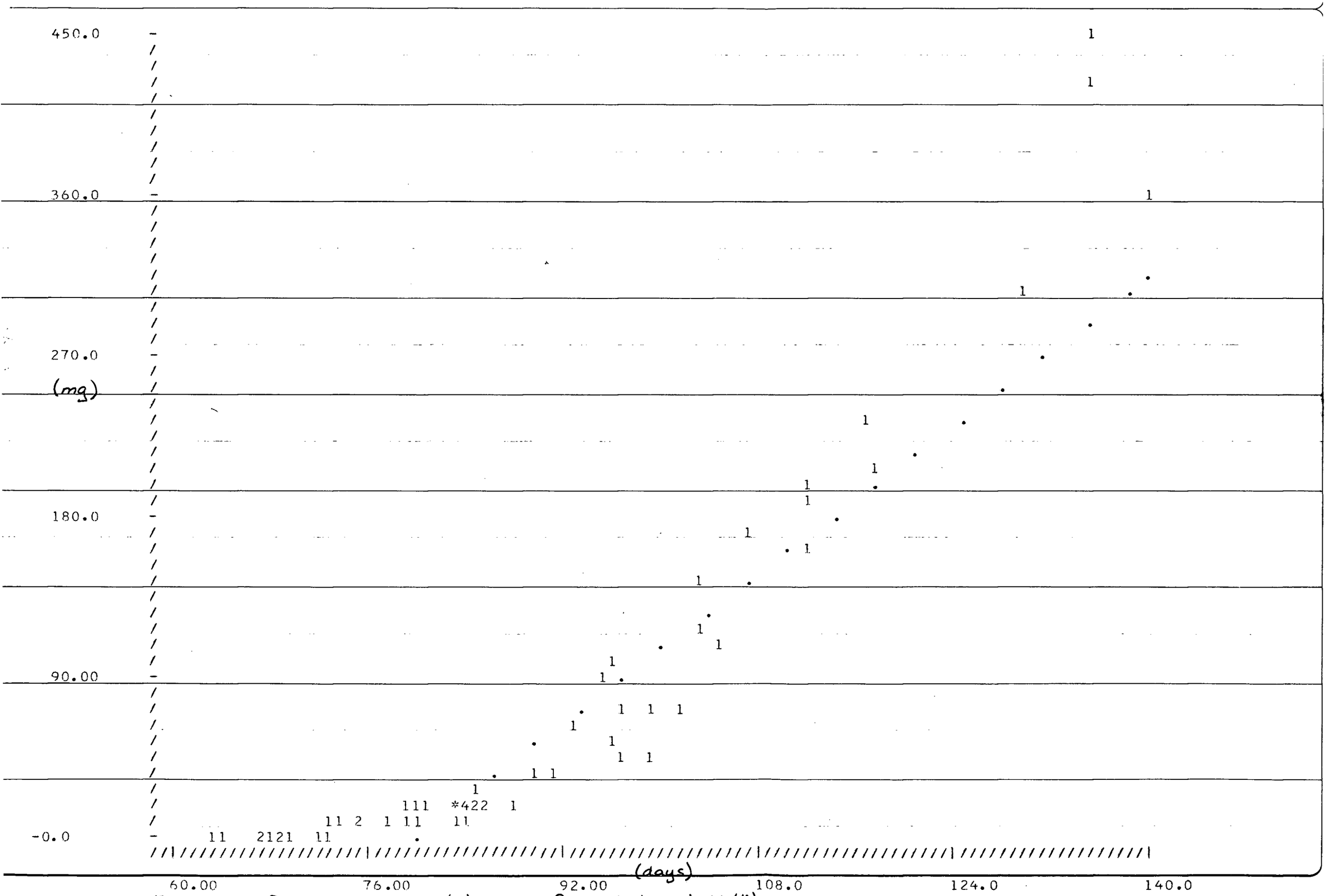

Exhibit 18. Developmental age (X) versus femoral dry weight (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
H-DRY	DEAGE	-269.4	3.472	482.4	0.0	14.94	0.1581	22.14	0.8960

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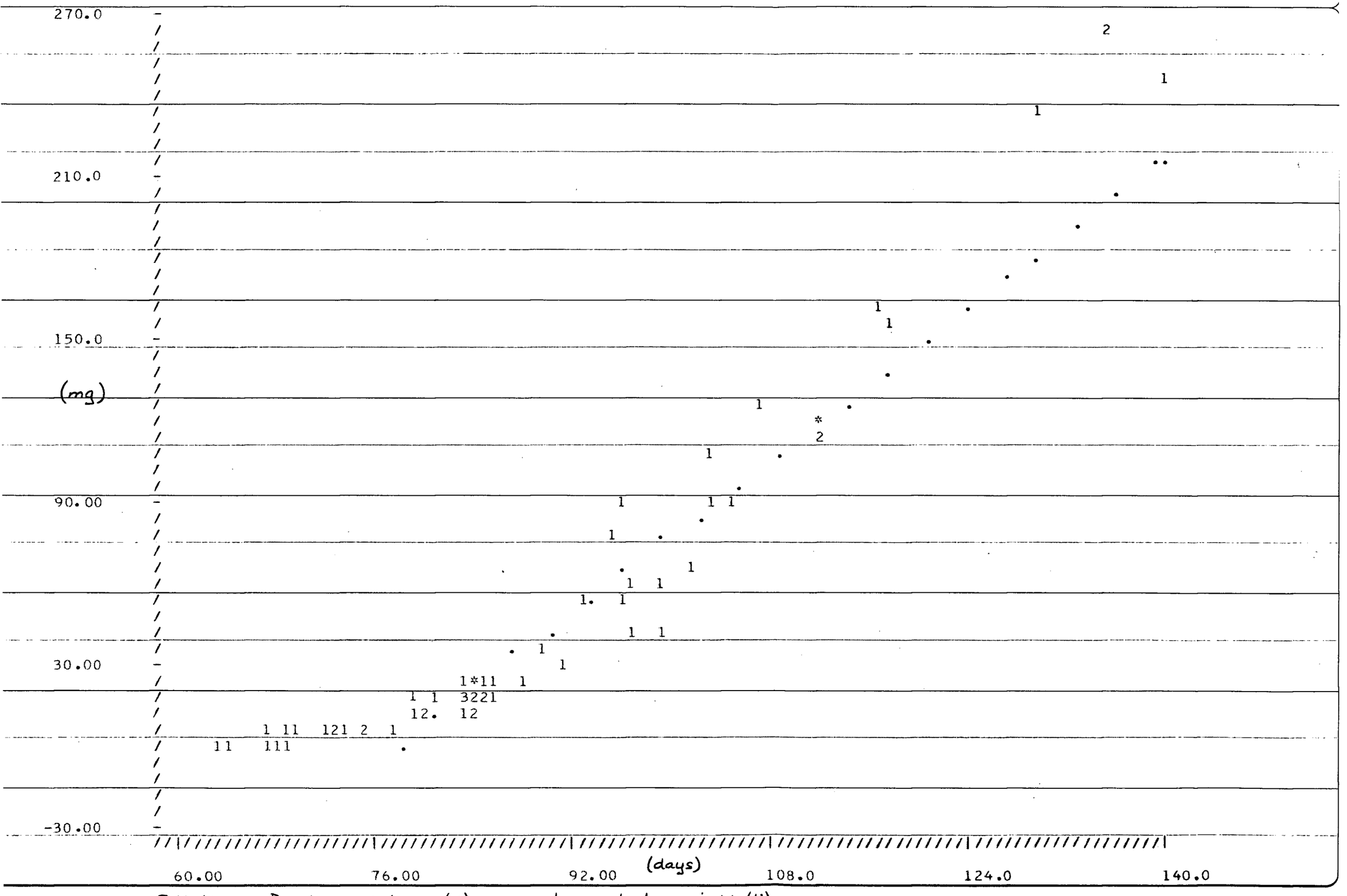

Exhibit 13. Developmental age (X) versus humeral dry weight (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
F-H20	DEAGE	102.3	-0.2224	256.2	0.0	1.314	0.1390D-01	1.946	0.8206

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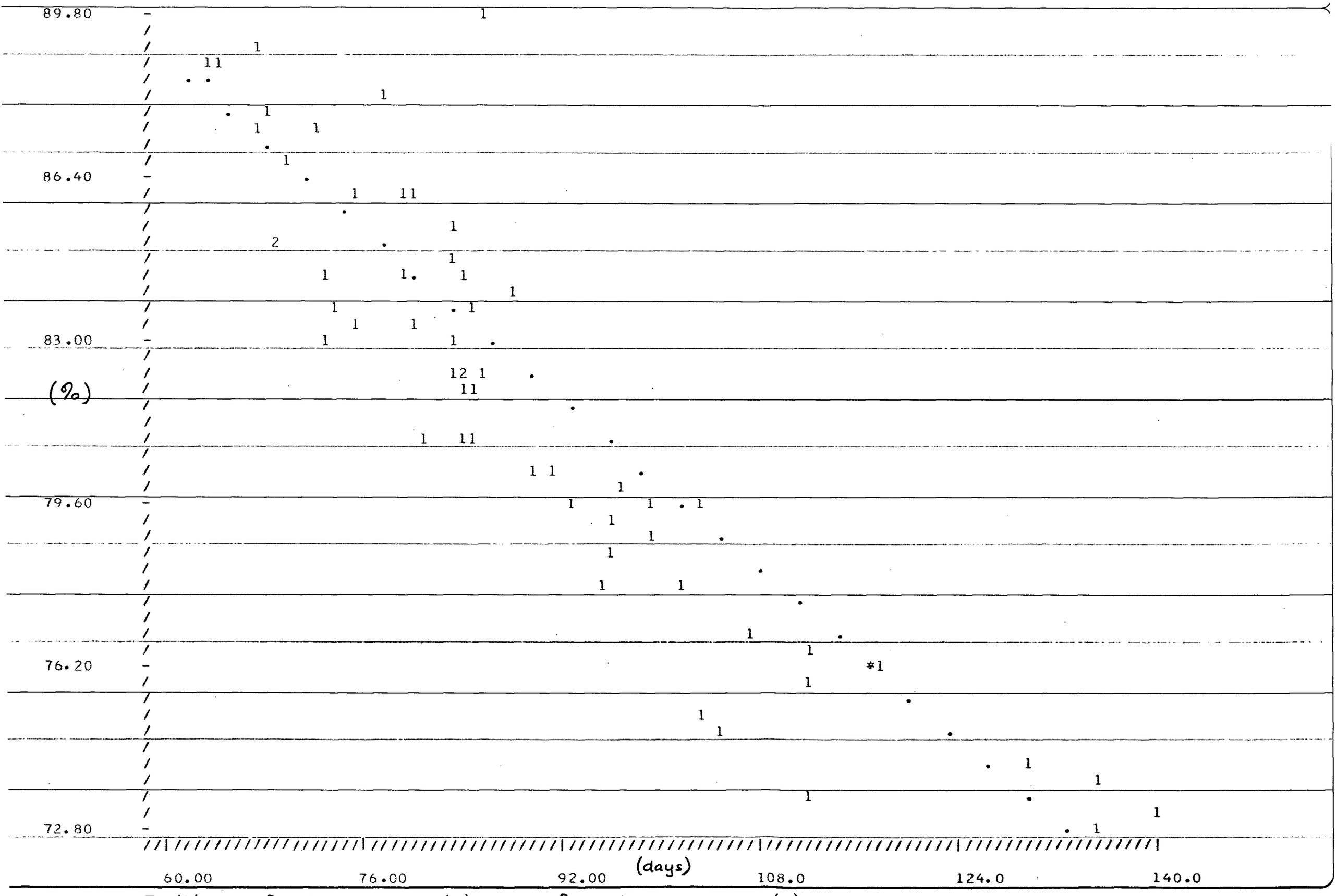

Exhibit 14. Developmental age (X) versus femoral water content (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
H-H2O	DEAGE	100.1	-0.2359	186.4	0.0000	1.633	0.1728D-01	2.419	0.7690

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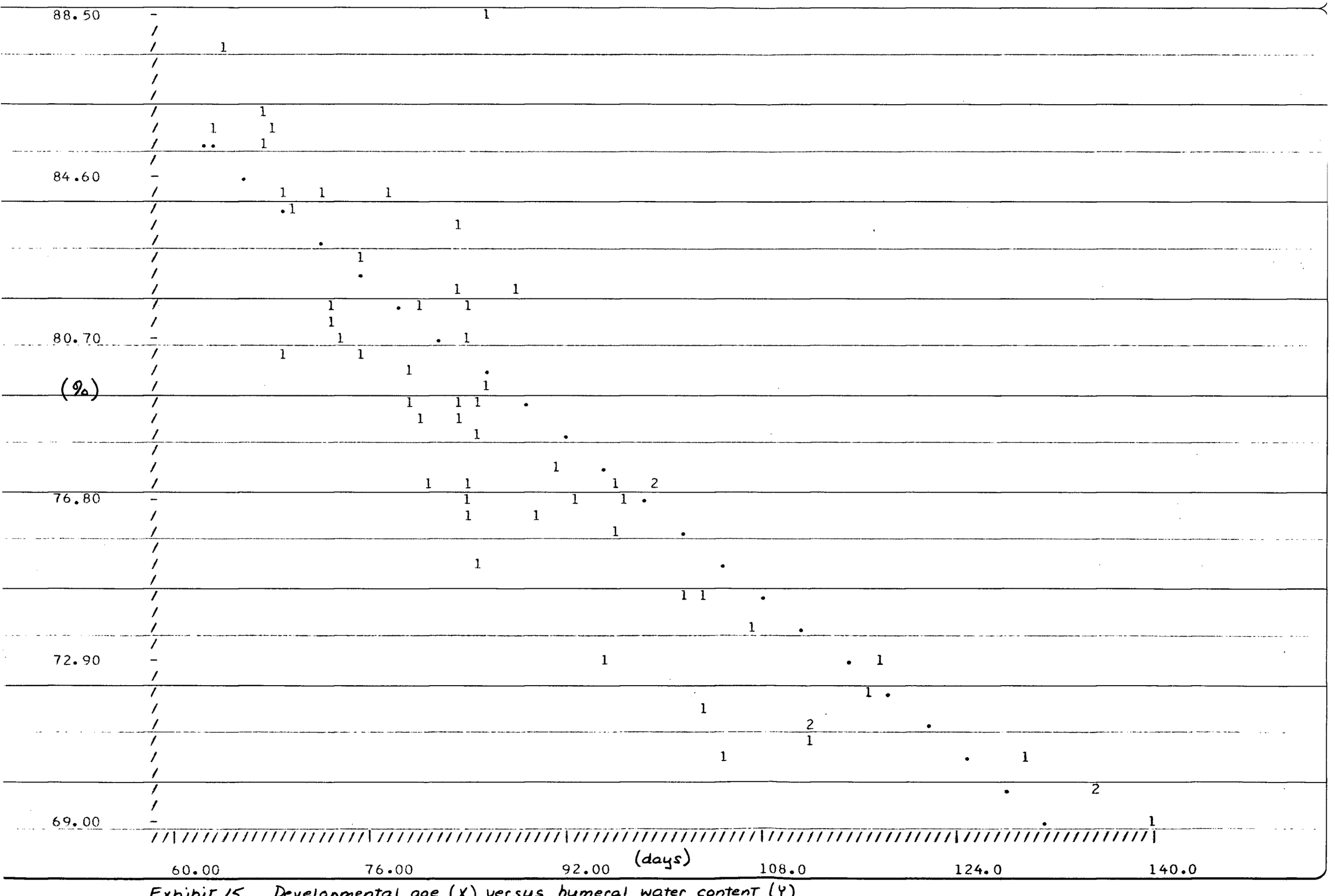

Exhibit 15. Developmental age (X) versus humeral water content (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
F-LEN	DEAGE	-27.65	0.5764	1044.	0.0	1.687	0.1784D-01	2.498	0.9491

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

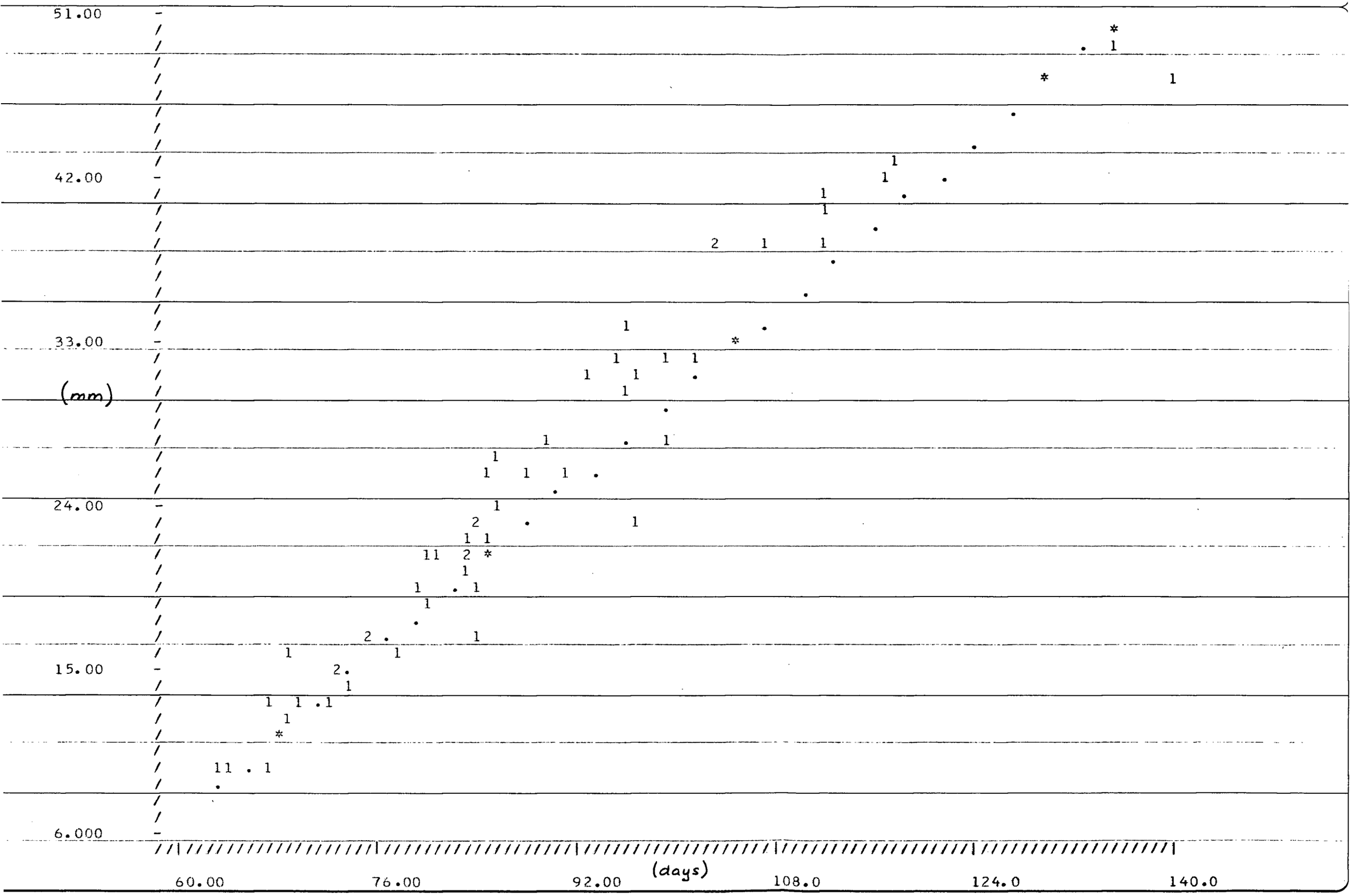

Exhibit 16. Developmental age (X) versus femoral length (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
H-LEN	DEAGE	-22.25	0.5002	966.6	0.0	1.521	0.1609D-01	2.253	0.9452

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

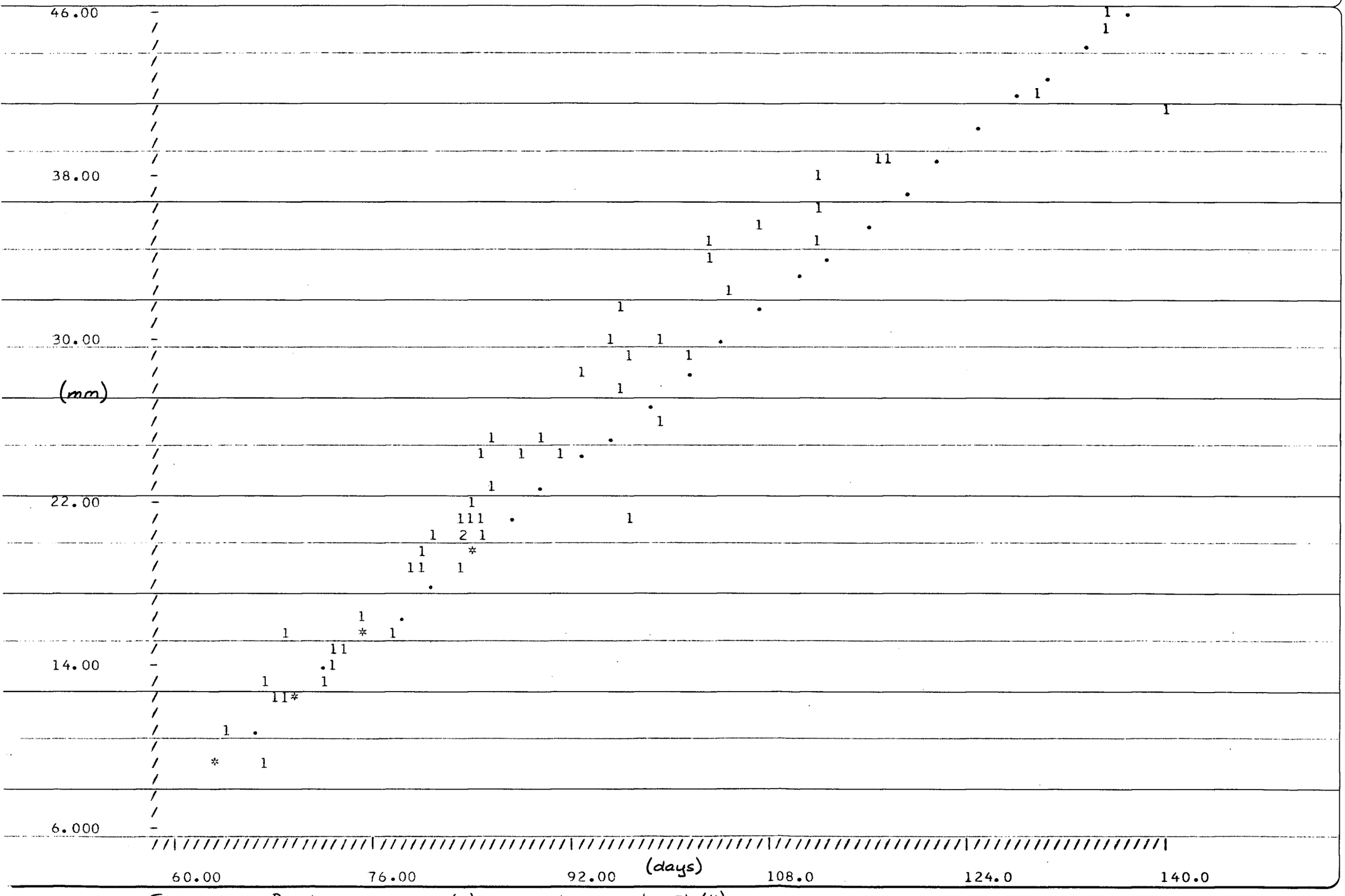

Exhibit 17. Developmental age (X) versus humeral length (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
F-OSS	DEAGE	-26.37	0.4574	1391.	0.0	1.160	0.1227D-01	1.718	0.9613

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

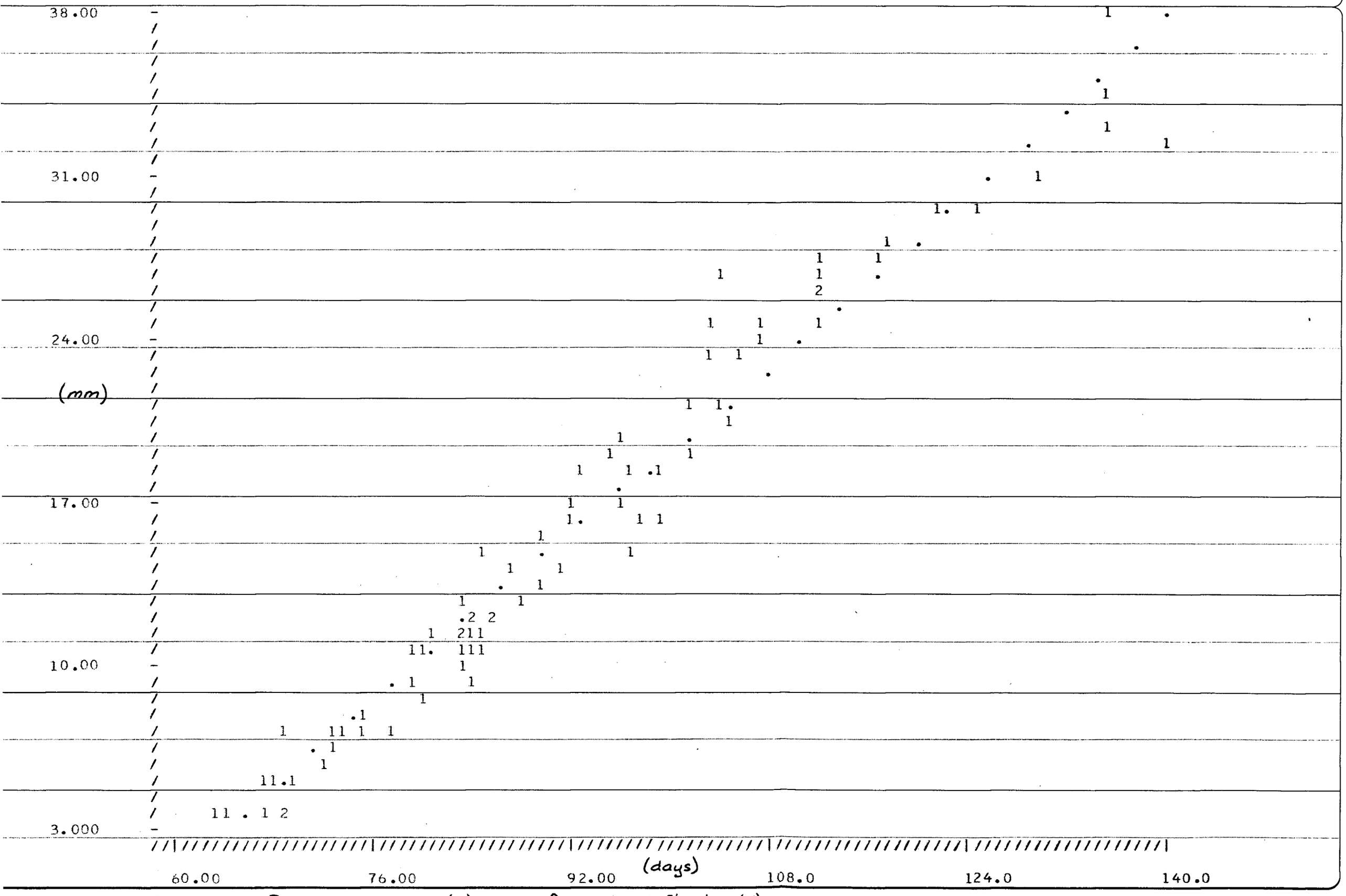

Exhibit 18. Developmental age (x) versus femoral ossification (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
H-OSS	DEAGE	-24.15	0.4366	1579.	0.0	1.039	0.1099D-01	1.539	0.9658

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

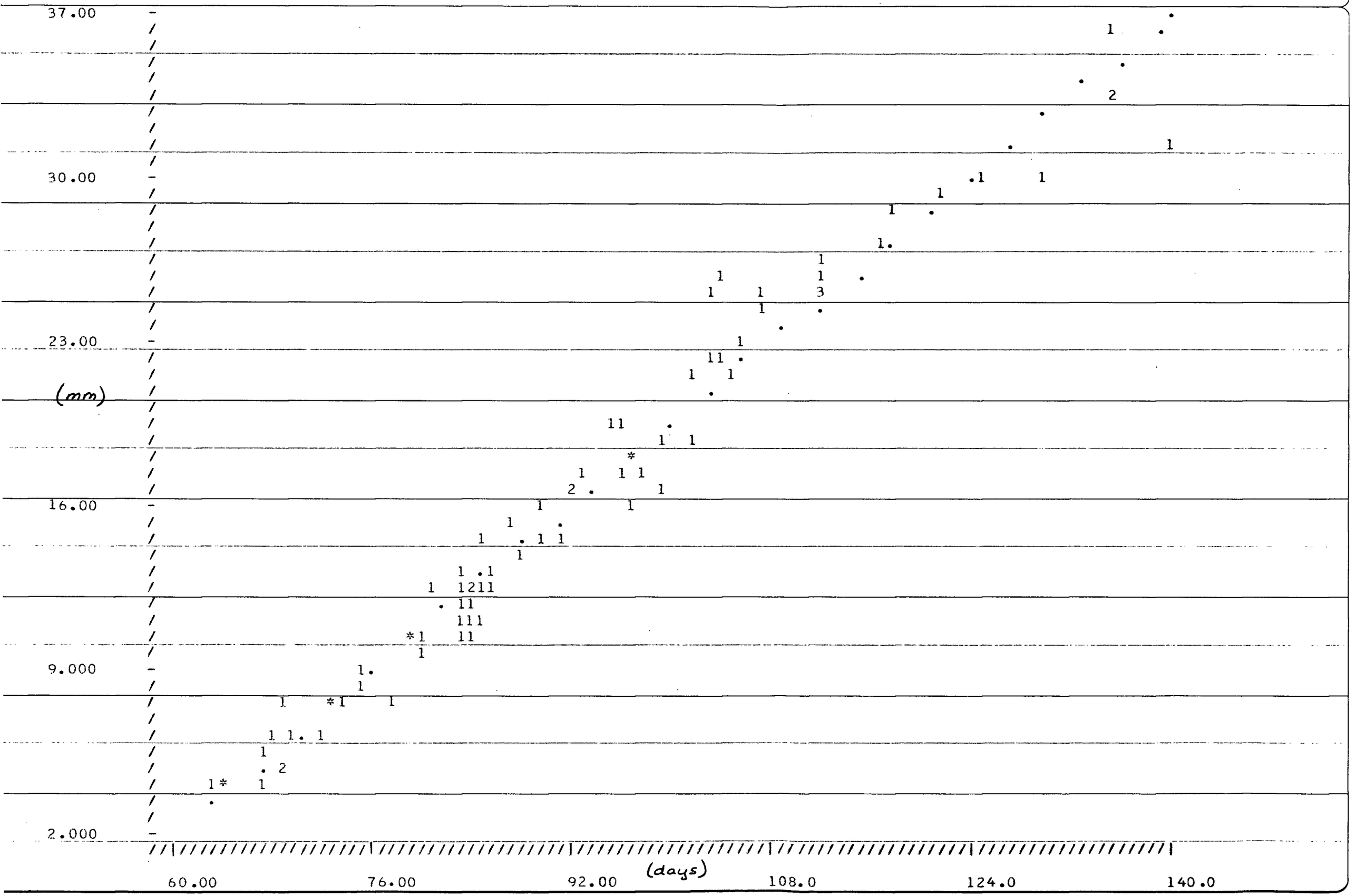

Exhibit 19. Developmental age (X) versus humeral ossification (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
F-COL	DEAGE	17.08	0.3623D-01	2.019	0.1571	2.410	0.2549D-01	3.570	0.0348

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

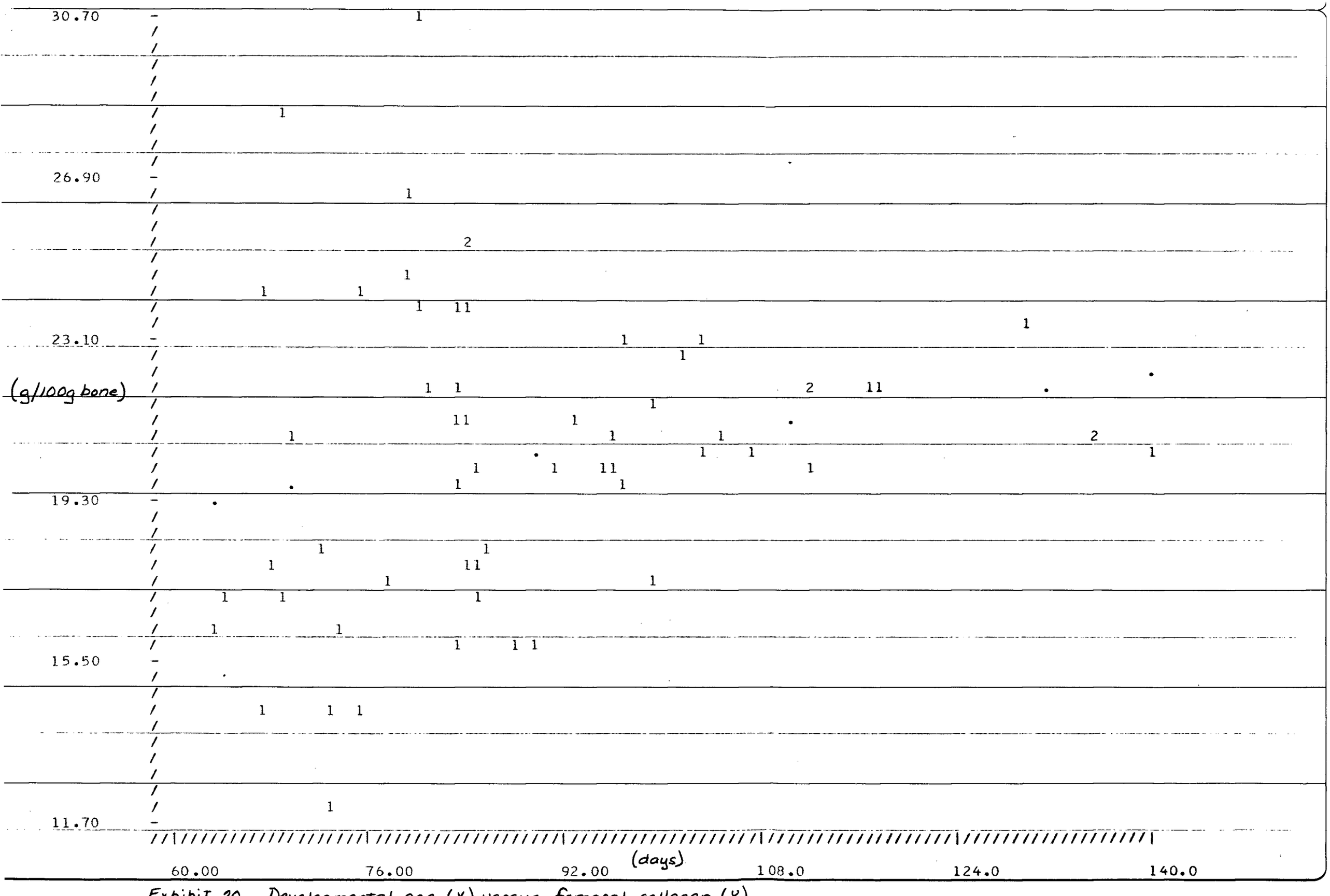

Exhibit 20. Developmental age (X) versus femoral collagen (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
H-COL	DEAGE	14.20	0.6403D-01	6.499	0.0130	2.374	0.2512D-01	3.517	0.1040

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

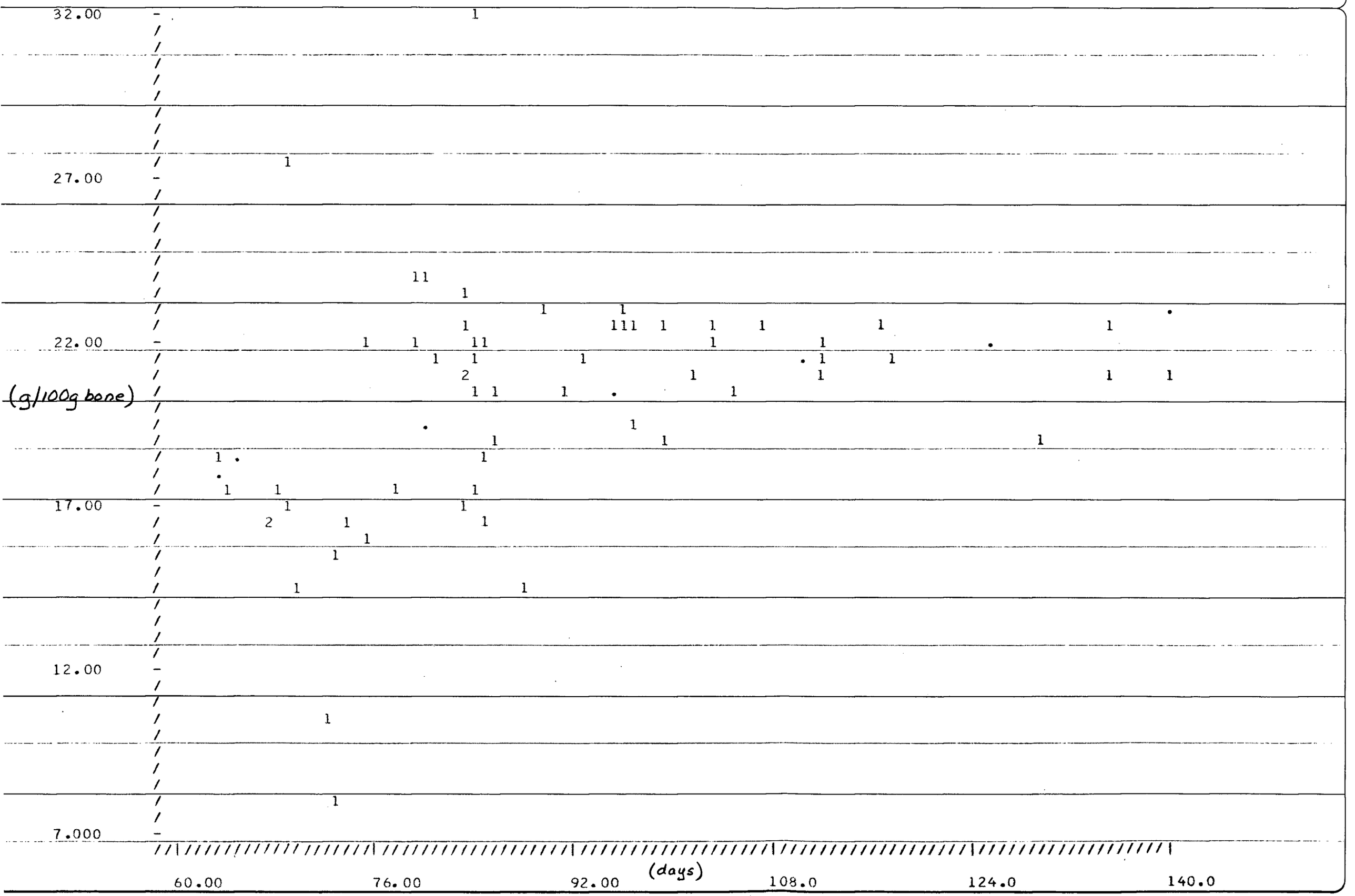

Exhibit 21. Developmental age (X) versus humeral collagen (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
F-CAL	DEAGE	-0.6499	0.1595	102.8	0.0000	1.487	0.1573D-01	2.202	0.6474

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

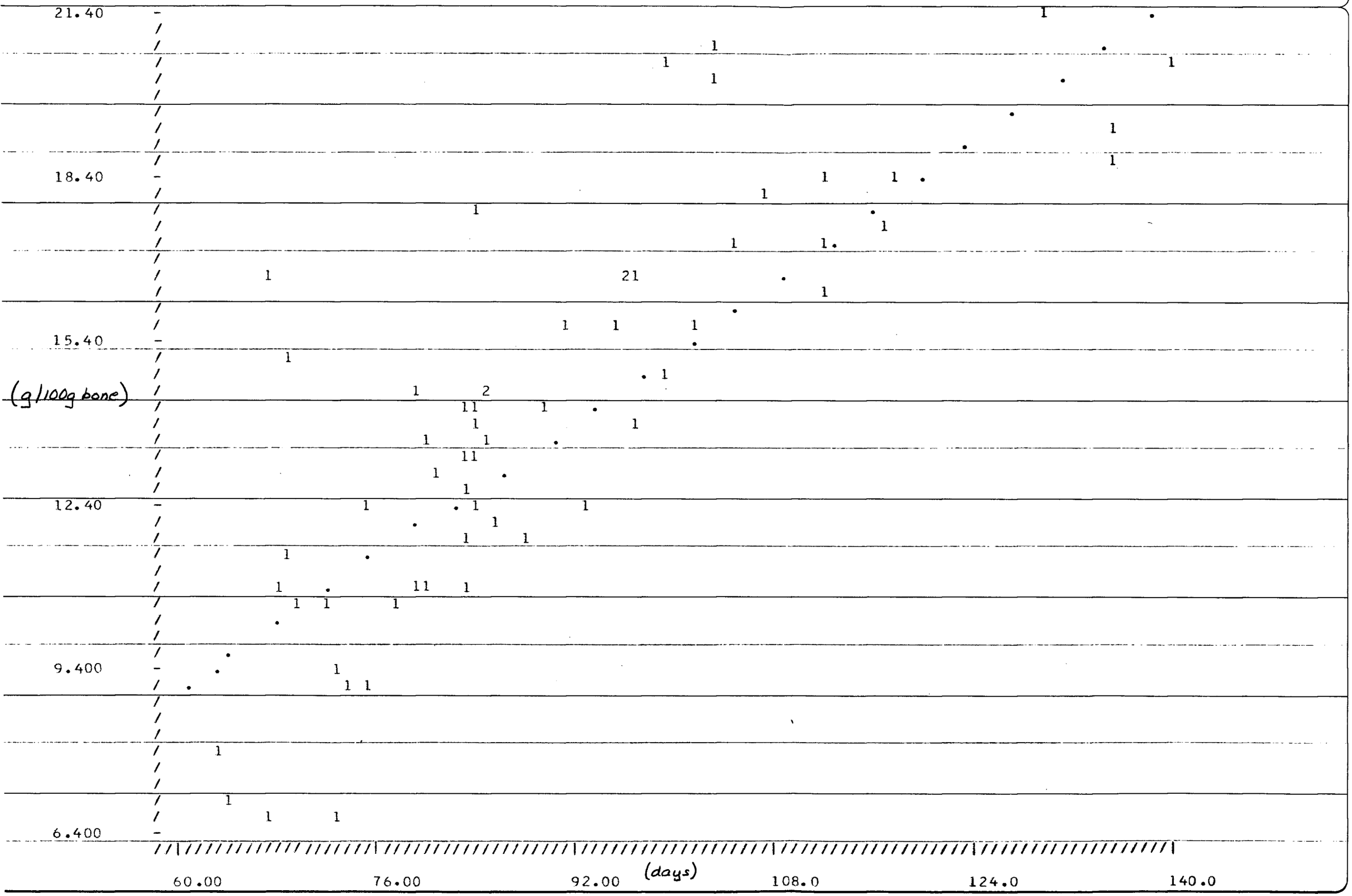

Exhibit 22. Developmental age (X) versus femoral calcium (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
H-CAL	DEAGE	-0.1232	0.1616	69.53	0.0000	1.832	0.1938D-01	2.713	0.5539

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

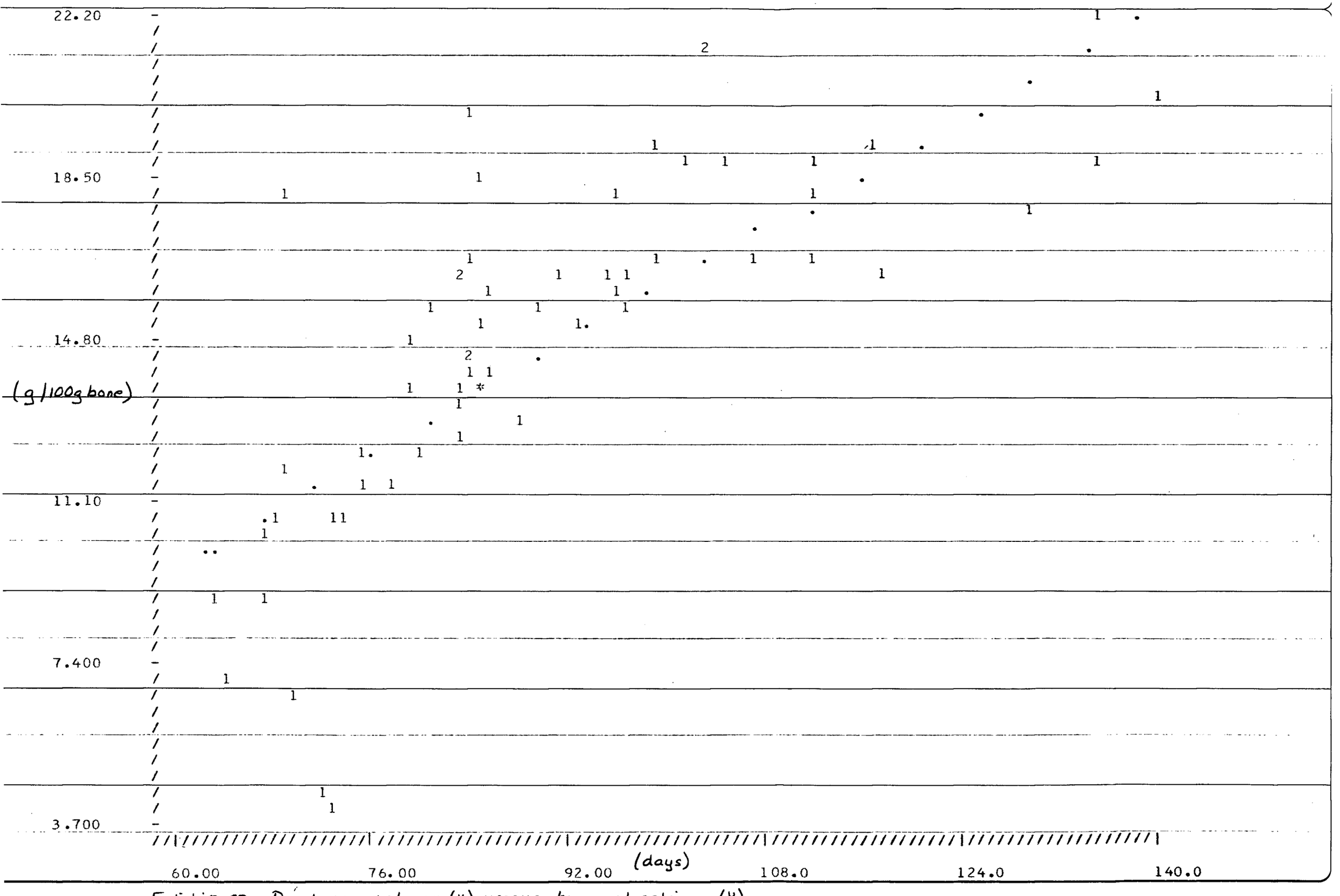

Exhibit 23. Developmental age (X) versus humeral calcium (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
F-PHO	DEAGE	2.708	0.4642D-01	44.77	0.0000	0.6558	0.6937D-02	0.9714	0.4443

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

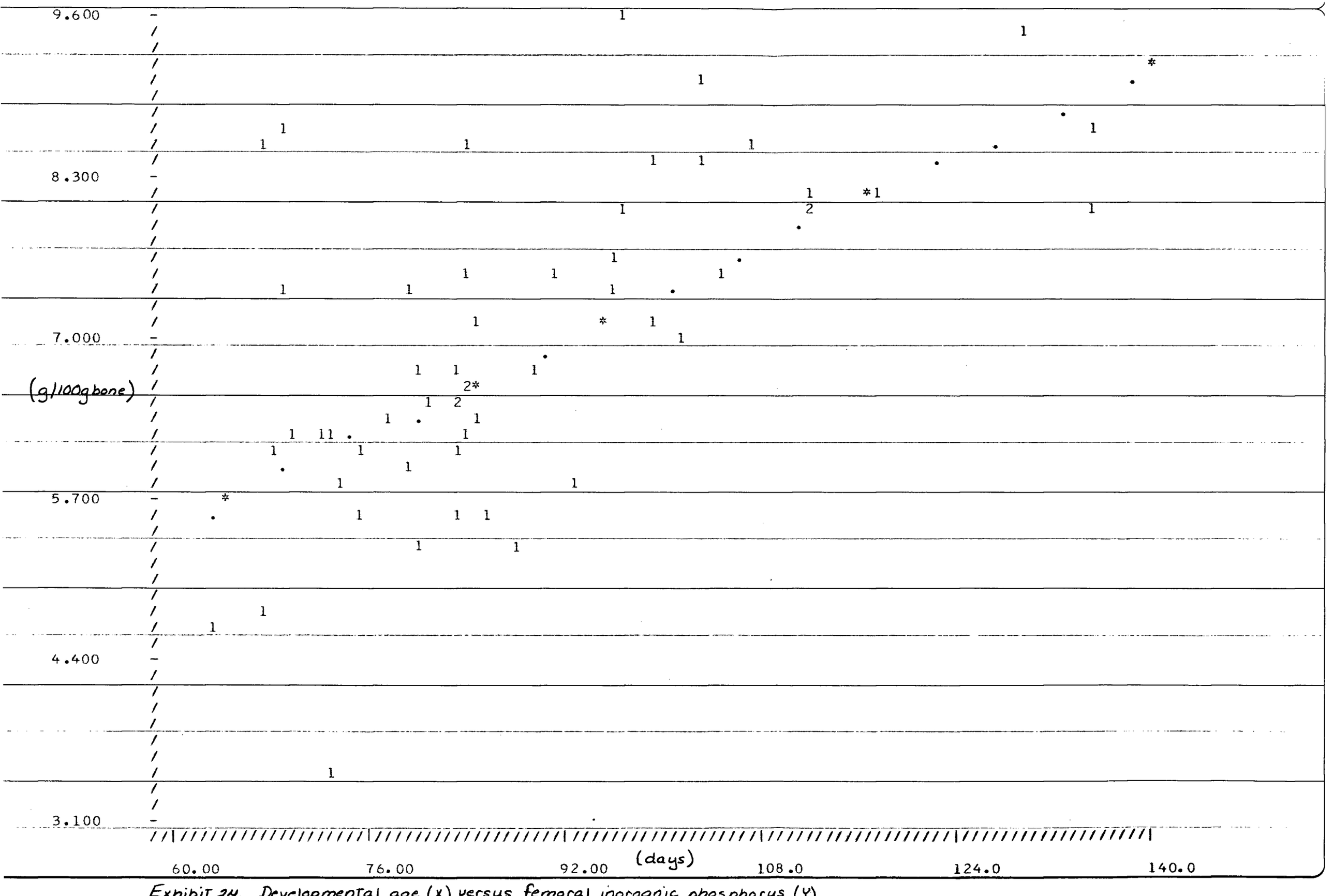

Exhibit 24. Developmental age (X) versus femoral inorganic phosphorus (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
H-PHO	DEAGE	2.902	0.4666D-01	31.17	0.0000	0.7900	0.8358D-02	1.170	0.3576

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

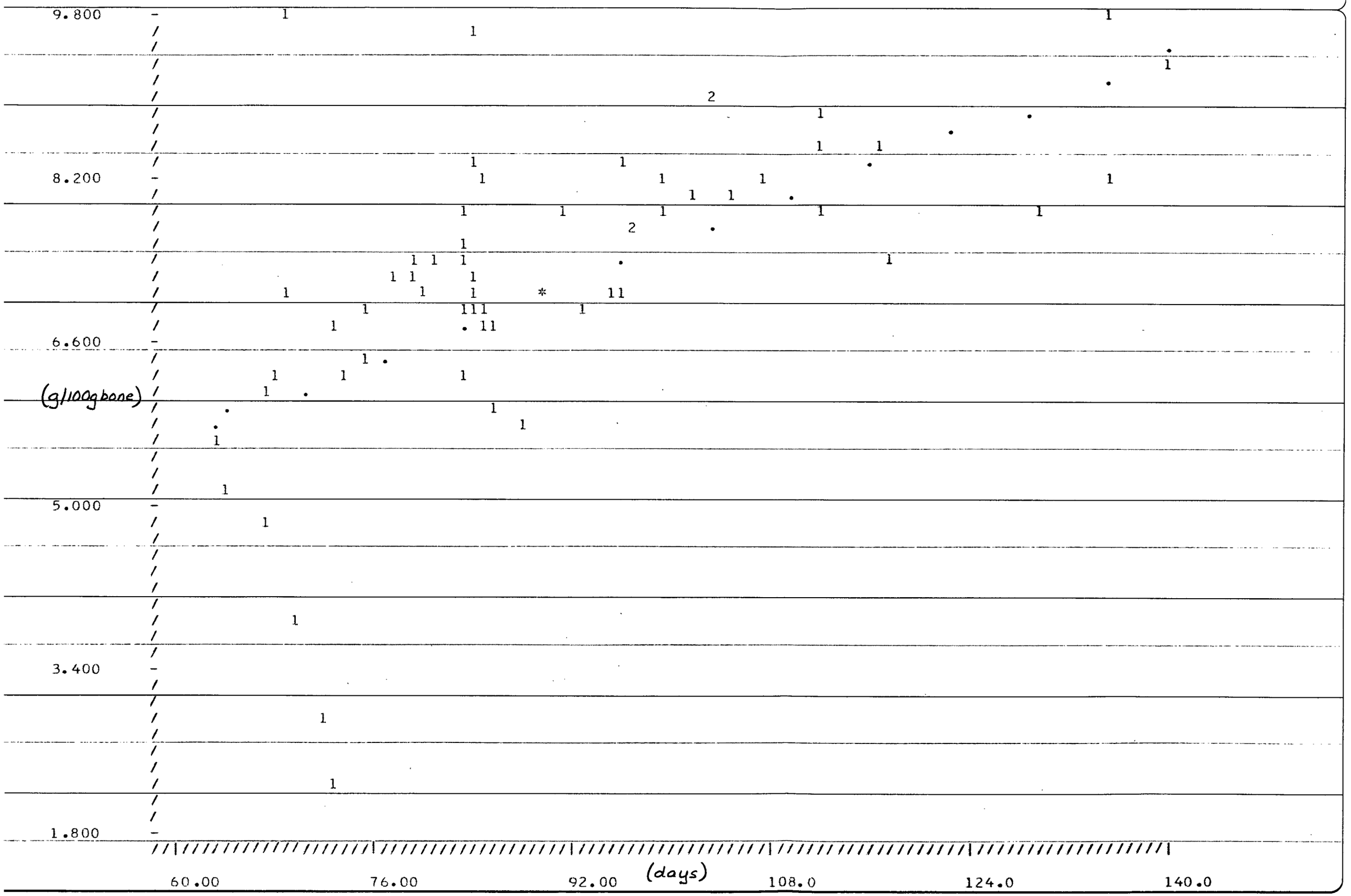

Exhibit #5. Developmental age (X) versus humeral inorganic phosphorus (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
F-MAG	DEAGE	0.6585	-0.1446D-02	3.965	0.0487	0.6864D-01	0.7262D-03	0.1017	0.0661

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

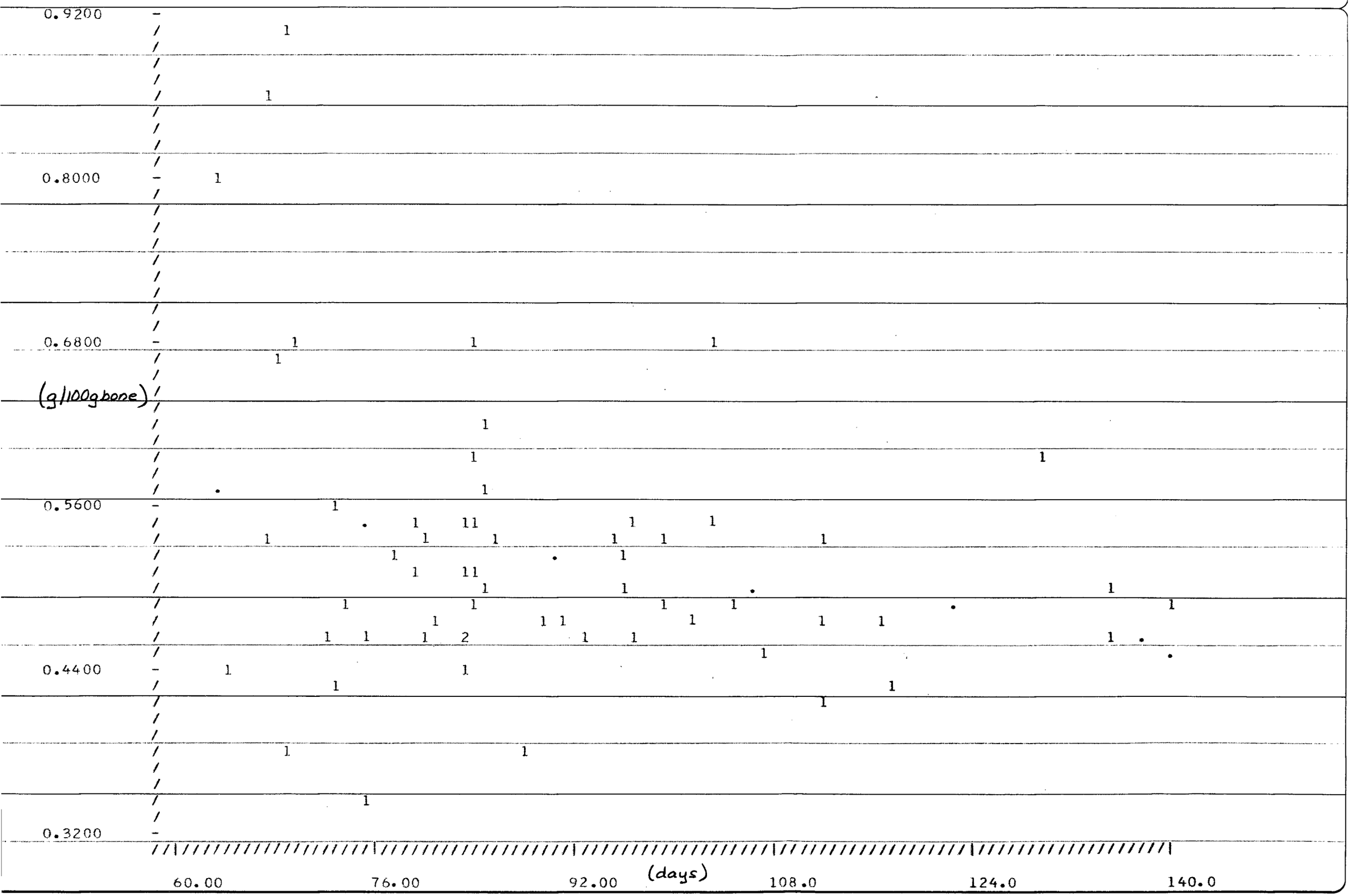

Exhibit 26. Developmental age (X) versus femoral magnesium (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
H-MAG	DEAGE	0.6890	-0.1542D-02	4.217	0.0424	0.7097D-01	0.7508D-03	0.1051	0.0700

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

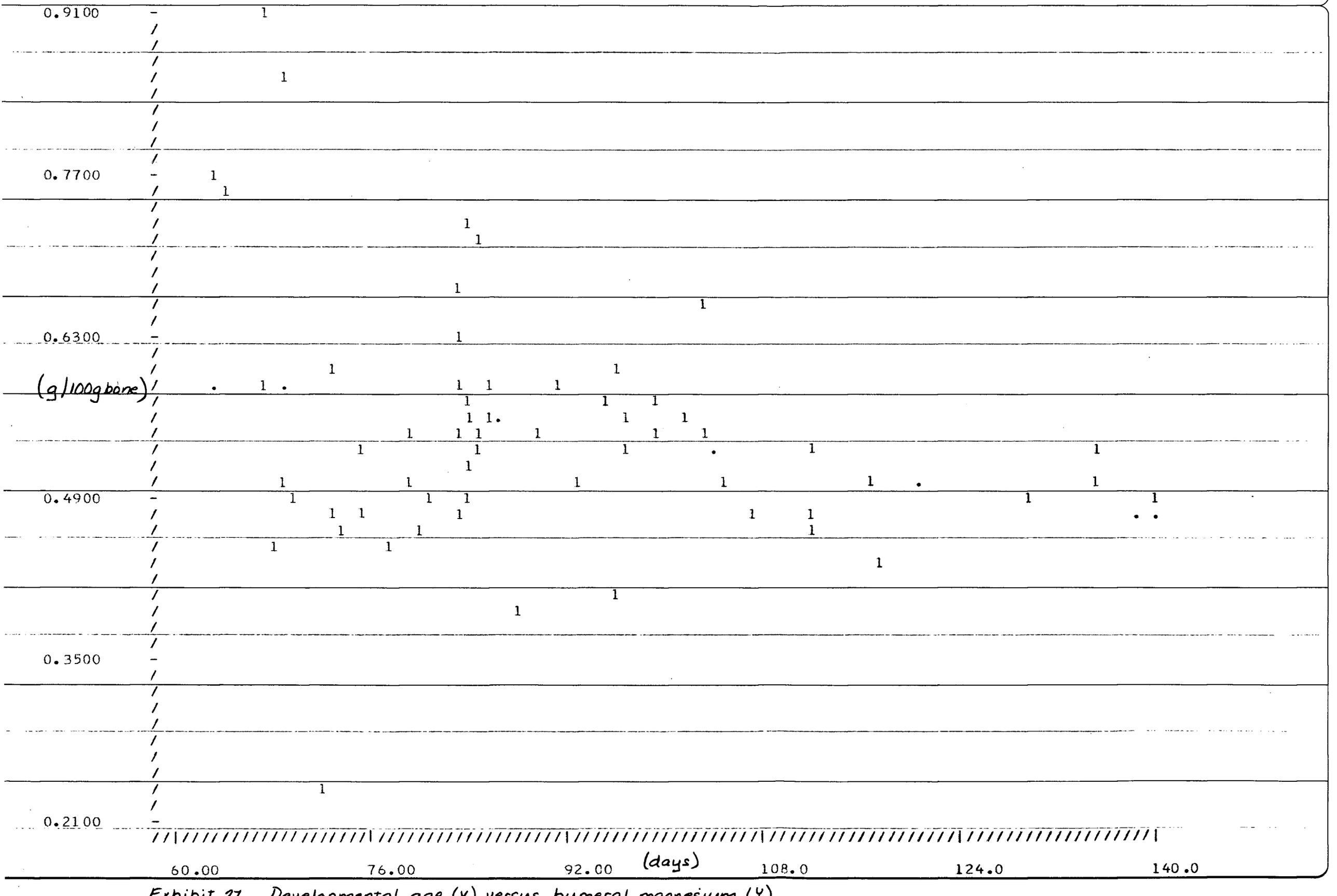

Exhibit 27. Developmental age (X) versus humeral magnesium (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
F-SOD	DEAGE	7.603	-0.6025D-01	29.72	0.0000	1.045	0.1105D-01	1.548	0.3467

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

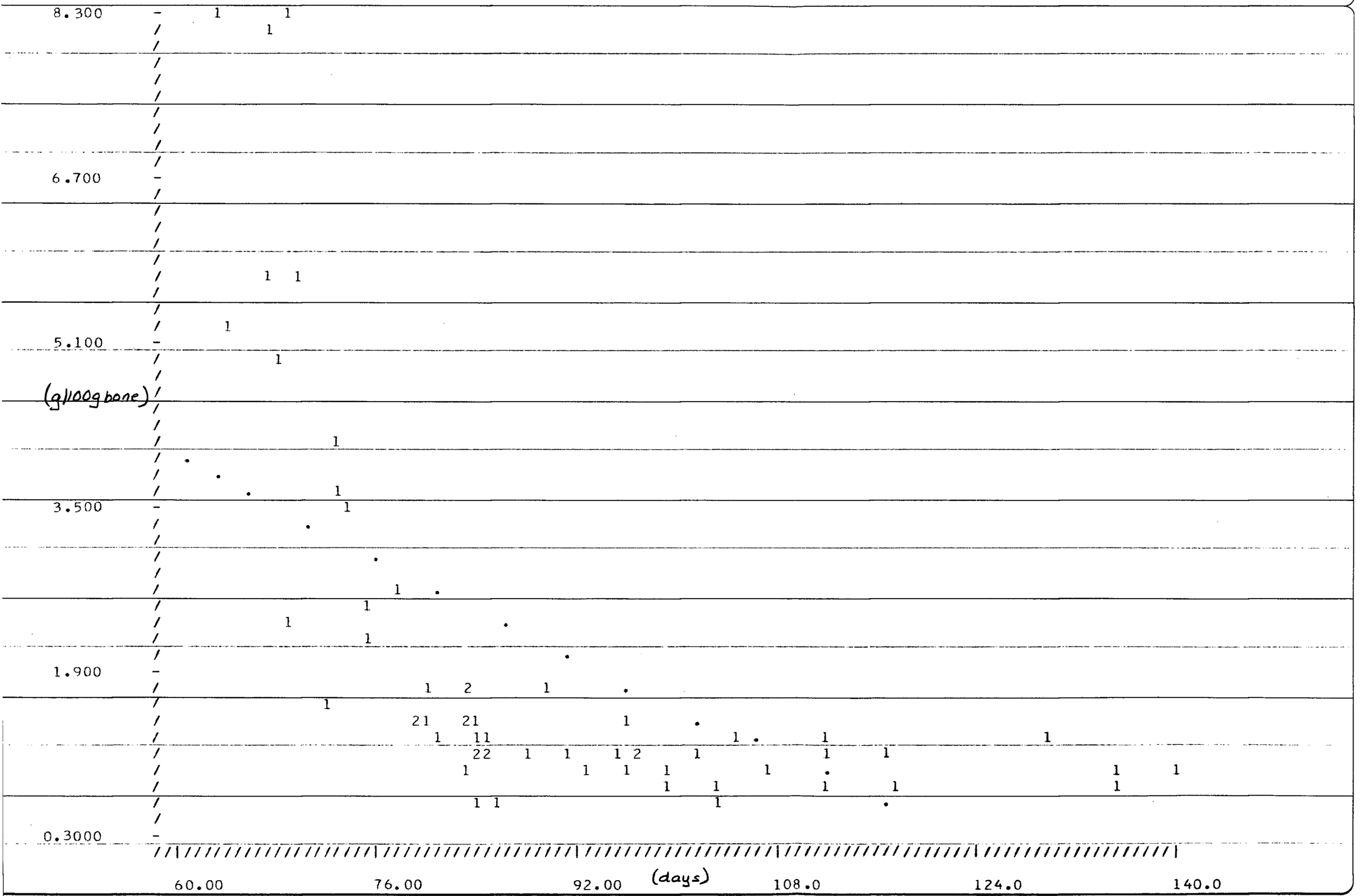

Exhibit 28. Developmental age (x) versus femoral sodium (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
H-SOD	DEAGE	6.424	-0.5045D-01	25.48	0.0000	0.9448	0.9994D-02	1.399	0.3127

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

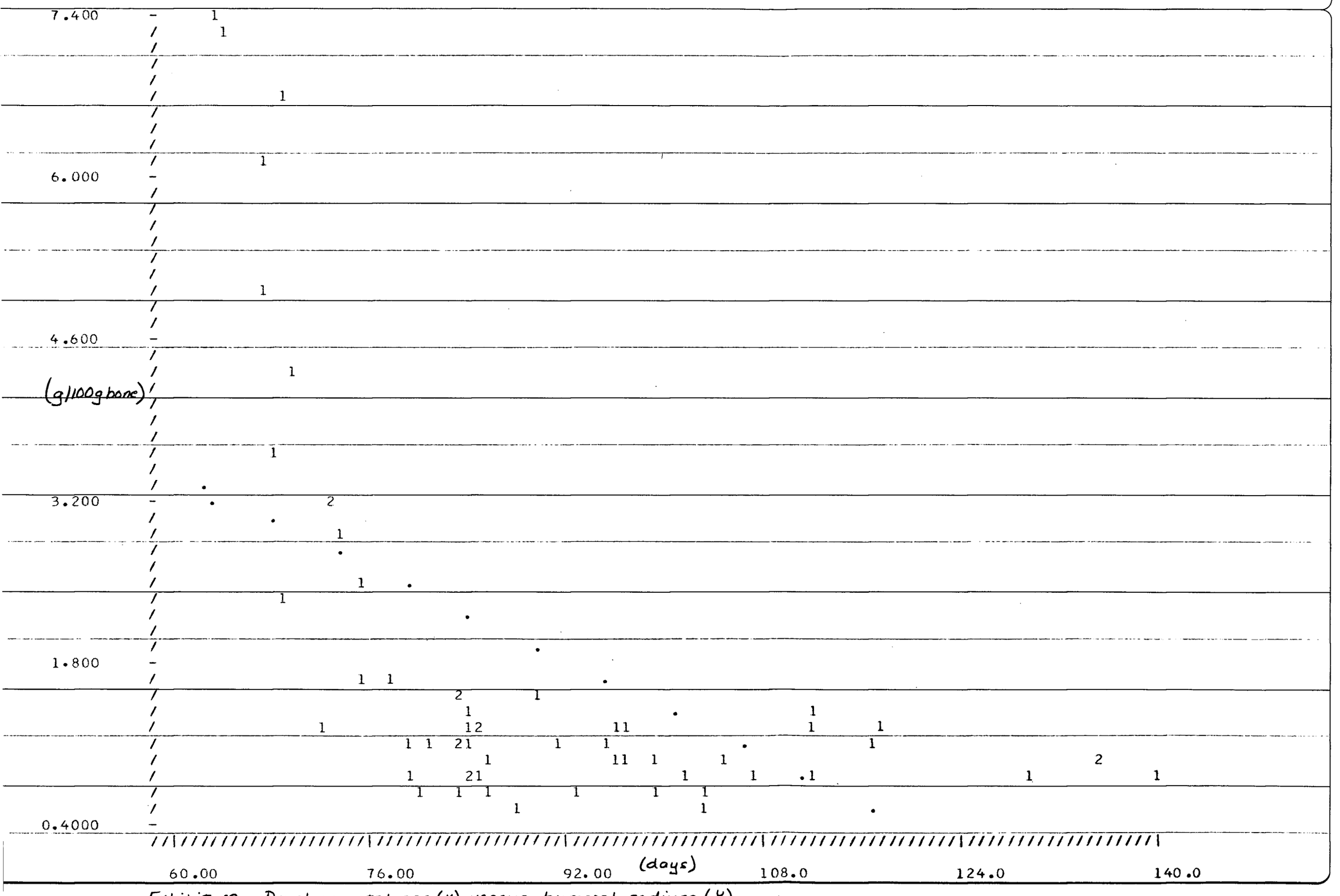

Exhibit 29. Developmental age (x) versus humeral sodium (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
FCACO	DEAGE	0.9567E-01	0.6479D-02	68.47	0.0000	0.7402D-01	0.7830D-03	0.1096	0.5501

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

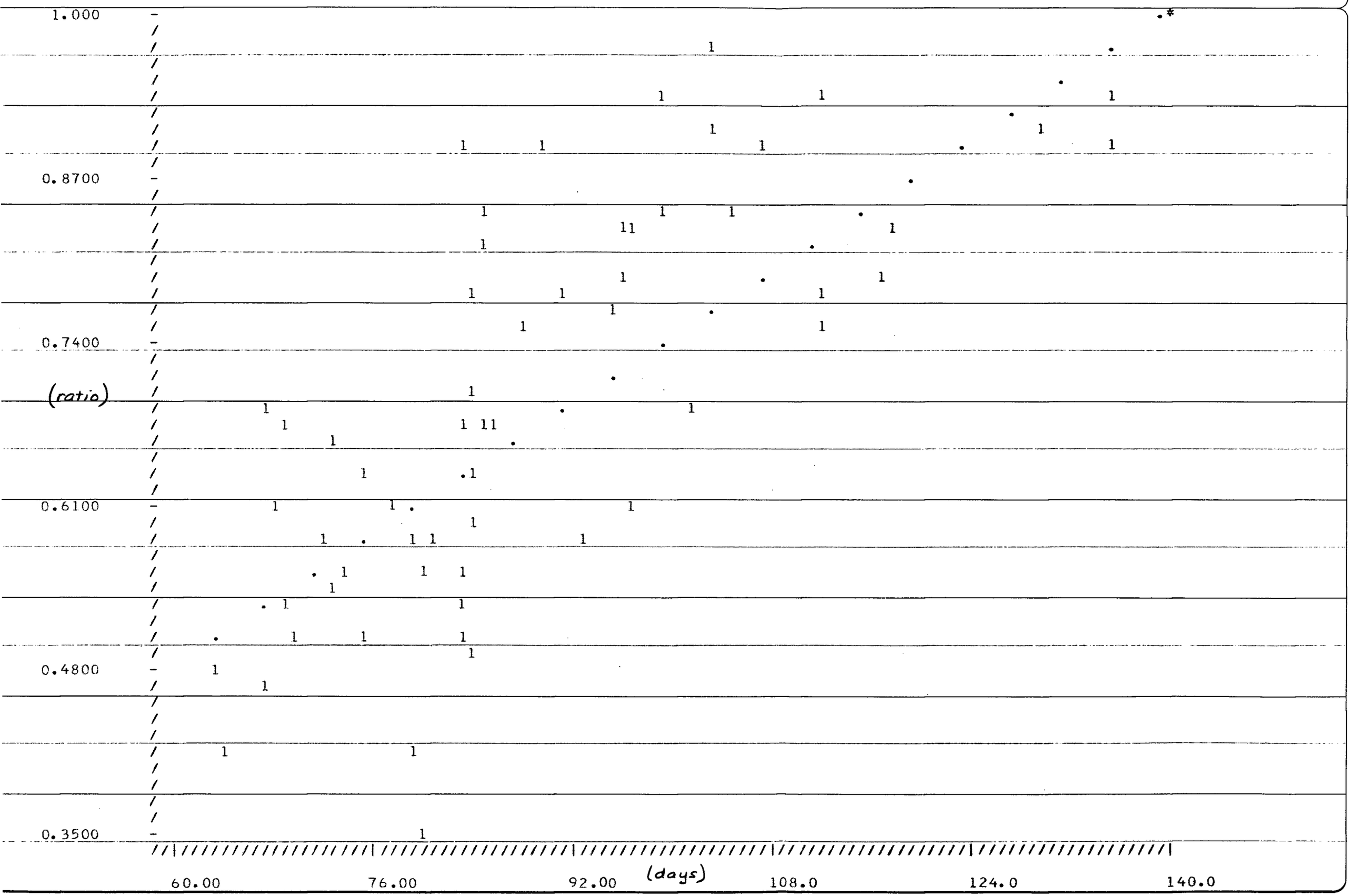

Exhibit 30. Developmental age (X) versus femoral calcium/collagen ratio (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
HCACO	DEAGE	0.1894	0.5872D-02	52.88	0.0000	0.7633D-01	0.8075D-03	0.1131	0.4857

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

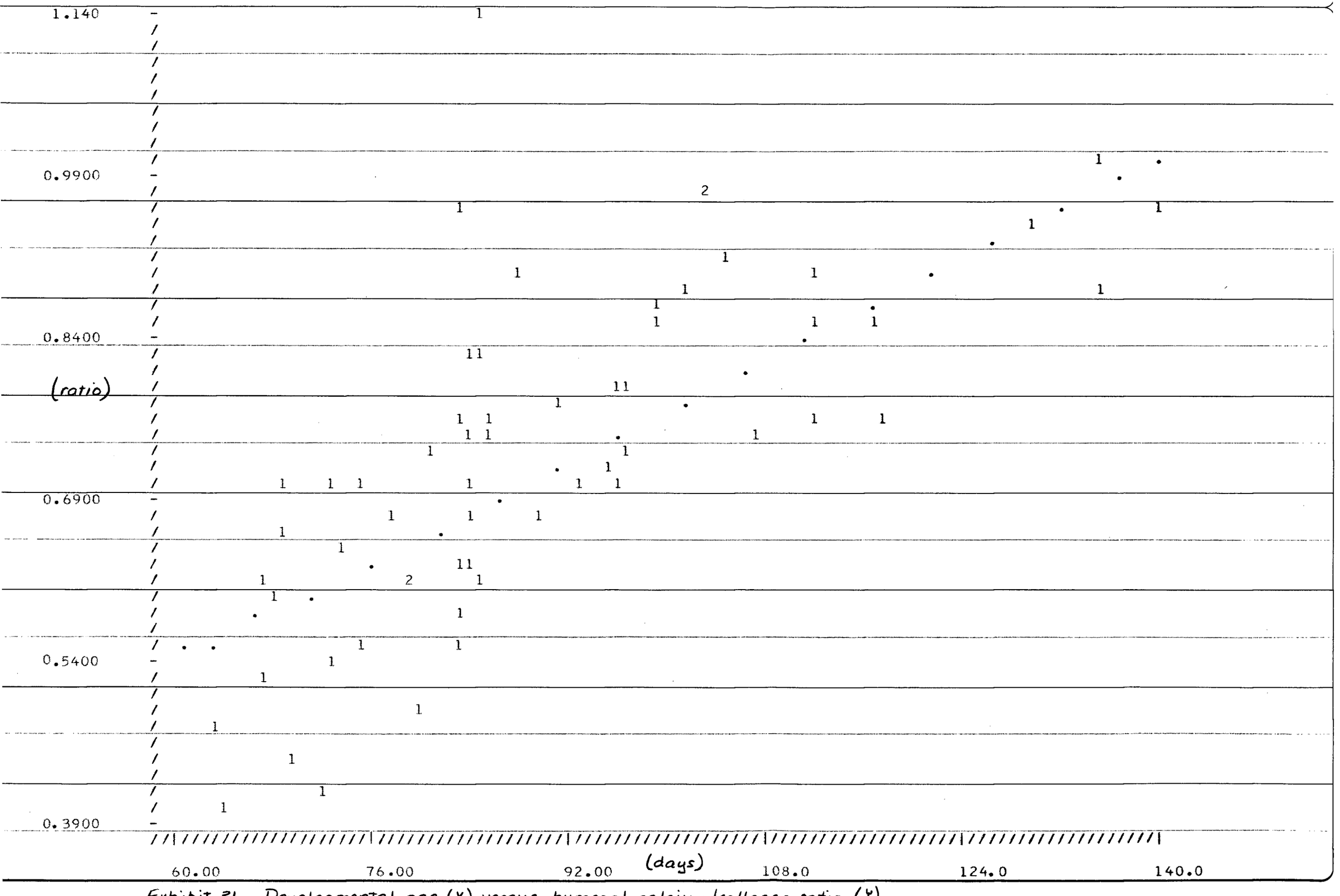

Exhibit 31. Developmental age (X) versus humeral calcium/collagen ratio (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
FCAPH	DEAGE	1.129	0.9384D-02	38.14	0.0000	0.1436	0.1519D-02	0.2128	0.4052

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

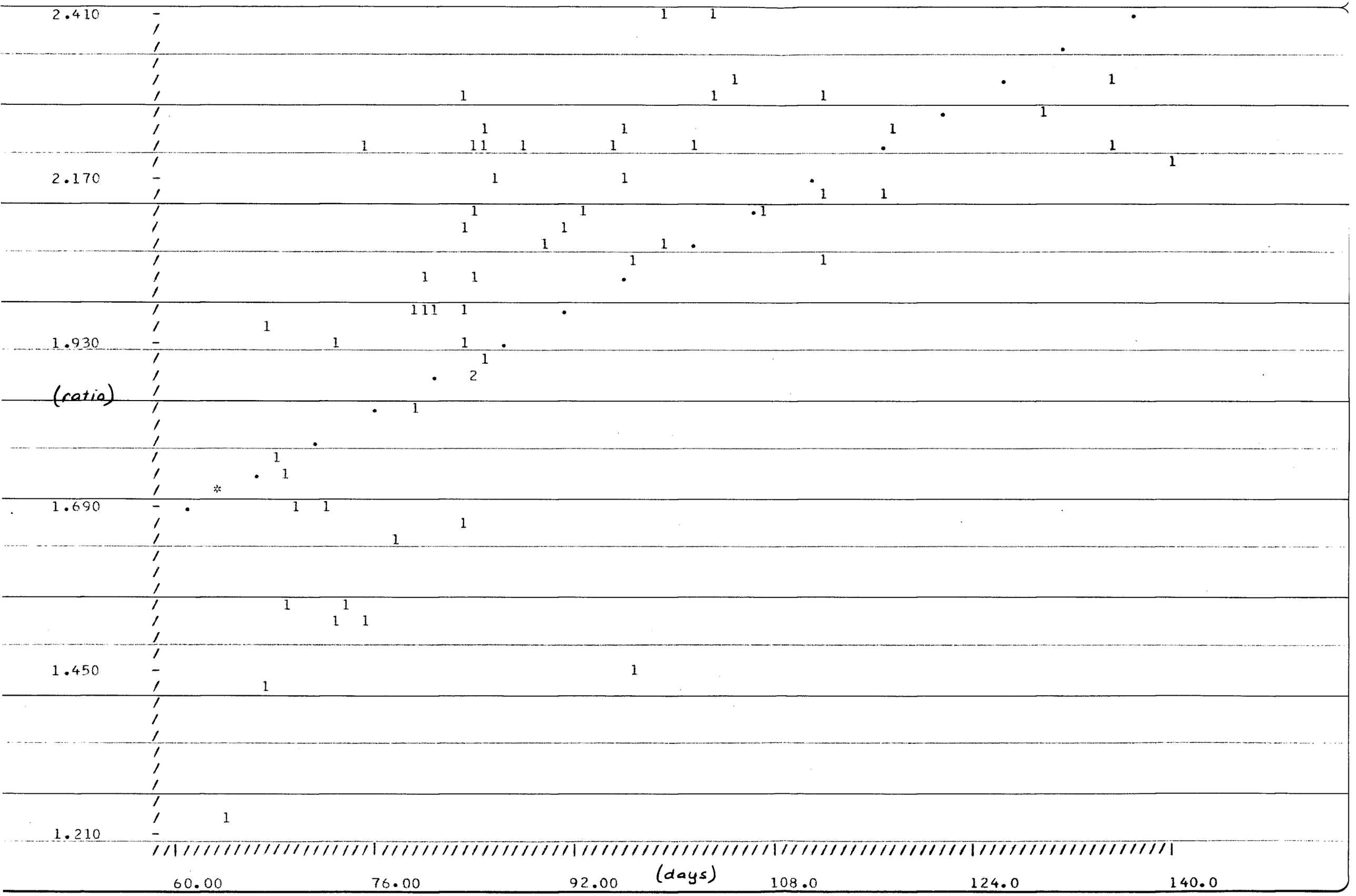

Exhibit 32. Developmental age (X) versus femoral calcium/phosphate ratio (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
HCAPH	DEAGE	1.131	0.9677D-02	51.62	0.0000	0.1273	0.1347D-02	0.1886	0.4796

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

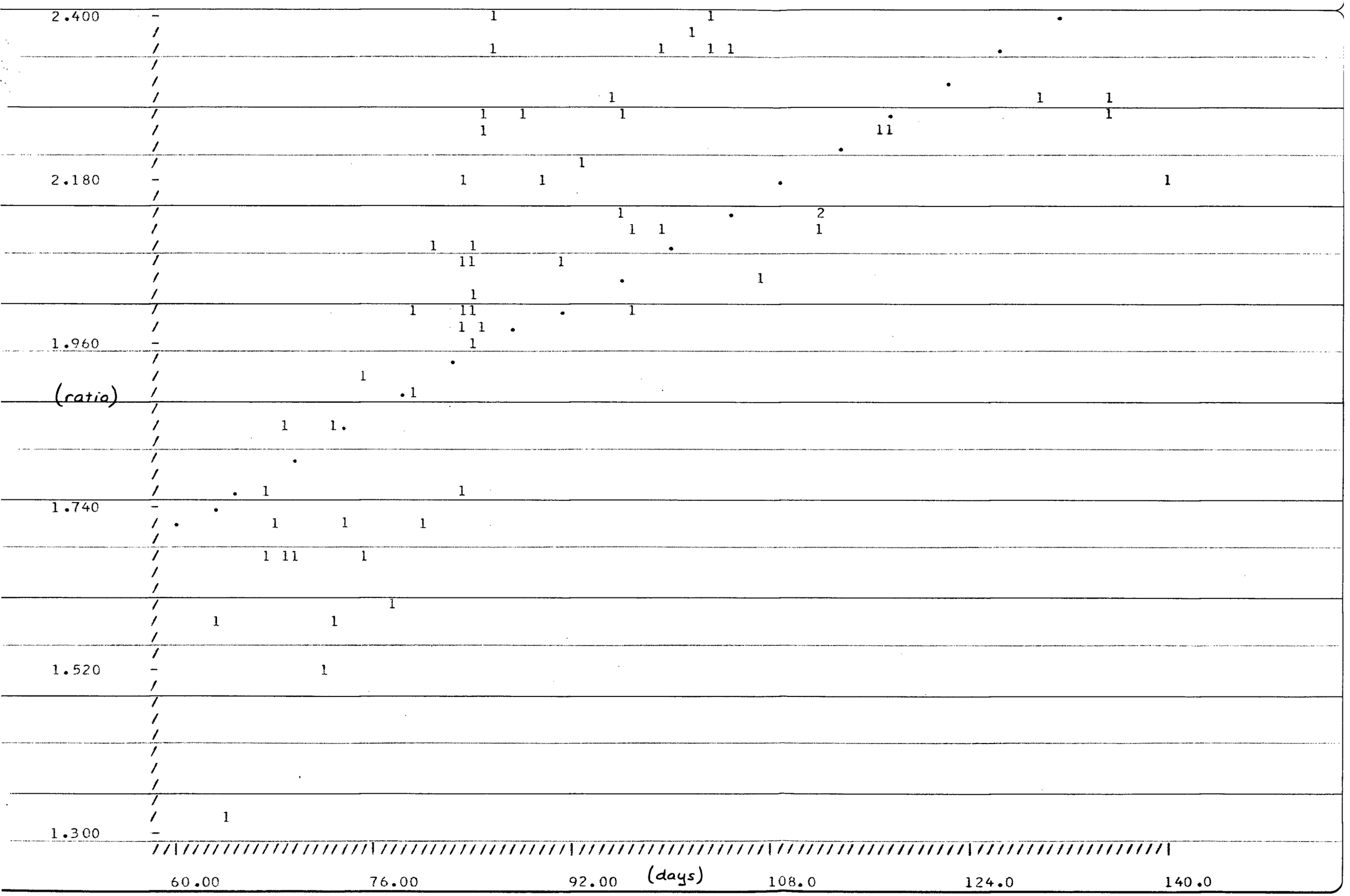

Exhibit 33. Developmental age (X) versus humeral calcium/phosphate ratio (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
M-WEI	M-HEI	-233.0	3.127	17.45	0.0001	122.6	0.7486	35.01	0.2376

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

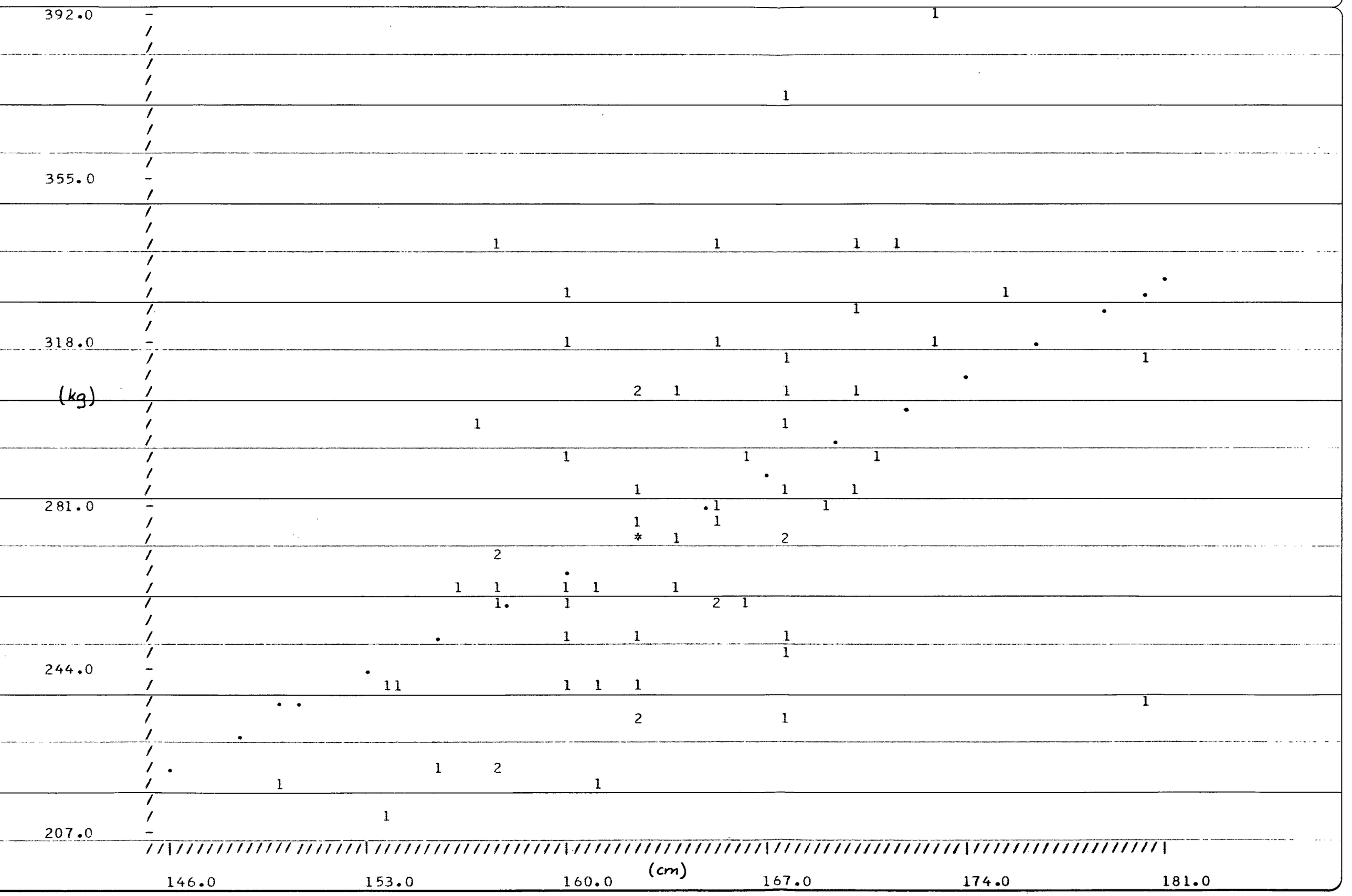

Exhibit 34. Maternal height (x) versus maternal weight (y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
M-PAR	M-AGE	-2.860	0.1641	50.04	0.0000	0.6952	0.2320D-01	1.463	0.4719

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

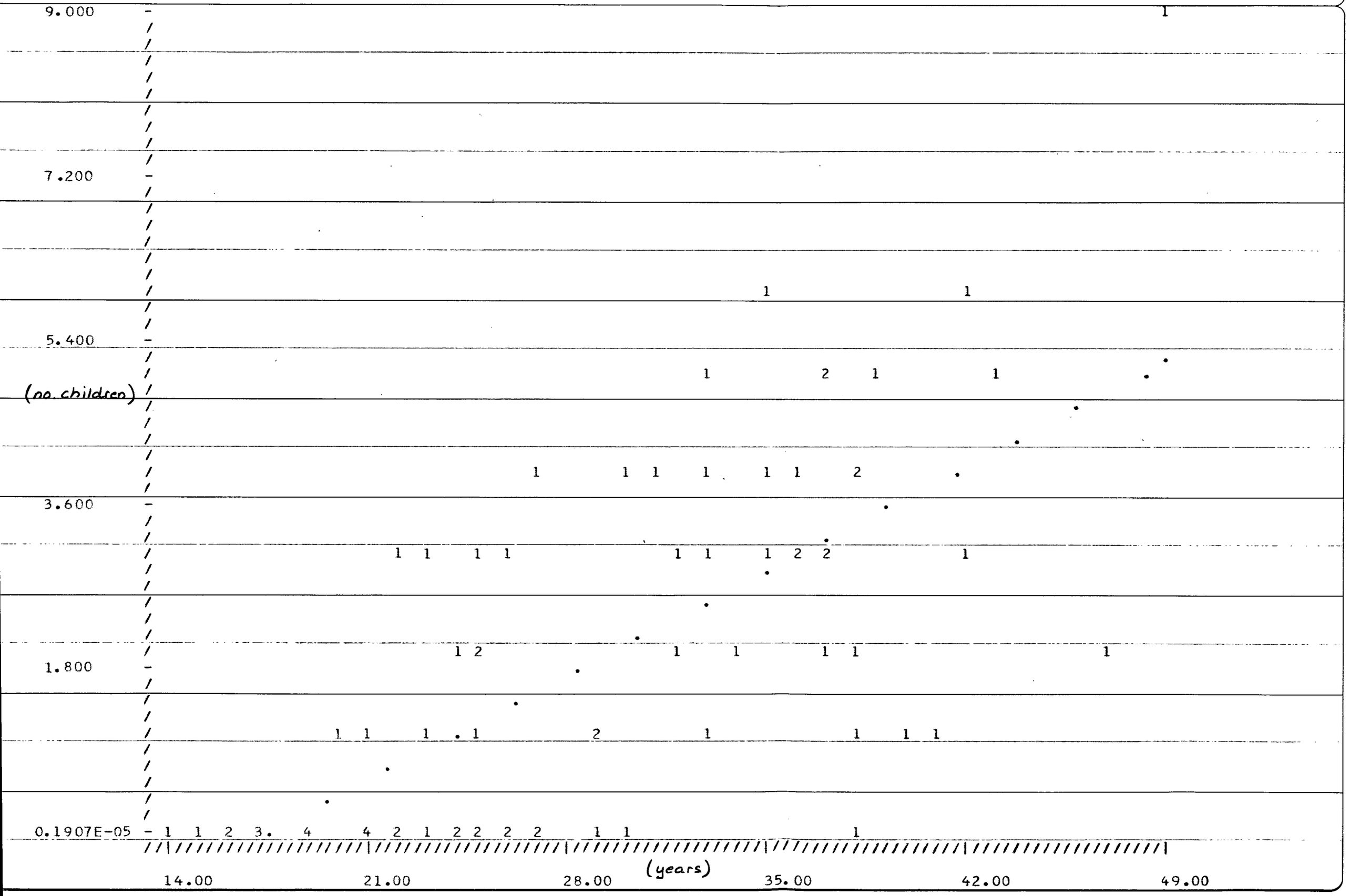

Exhibit 35. Maternal age (X) versus maternal parity (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
M-GRA	M-AGE	-2.213	0.1912	55.40	0.0000	0.7698	0.2569D-01	1.620	0.4973

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

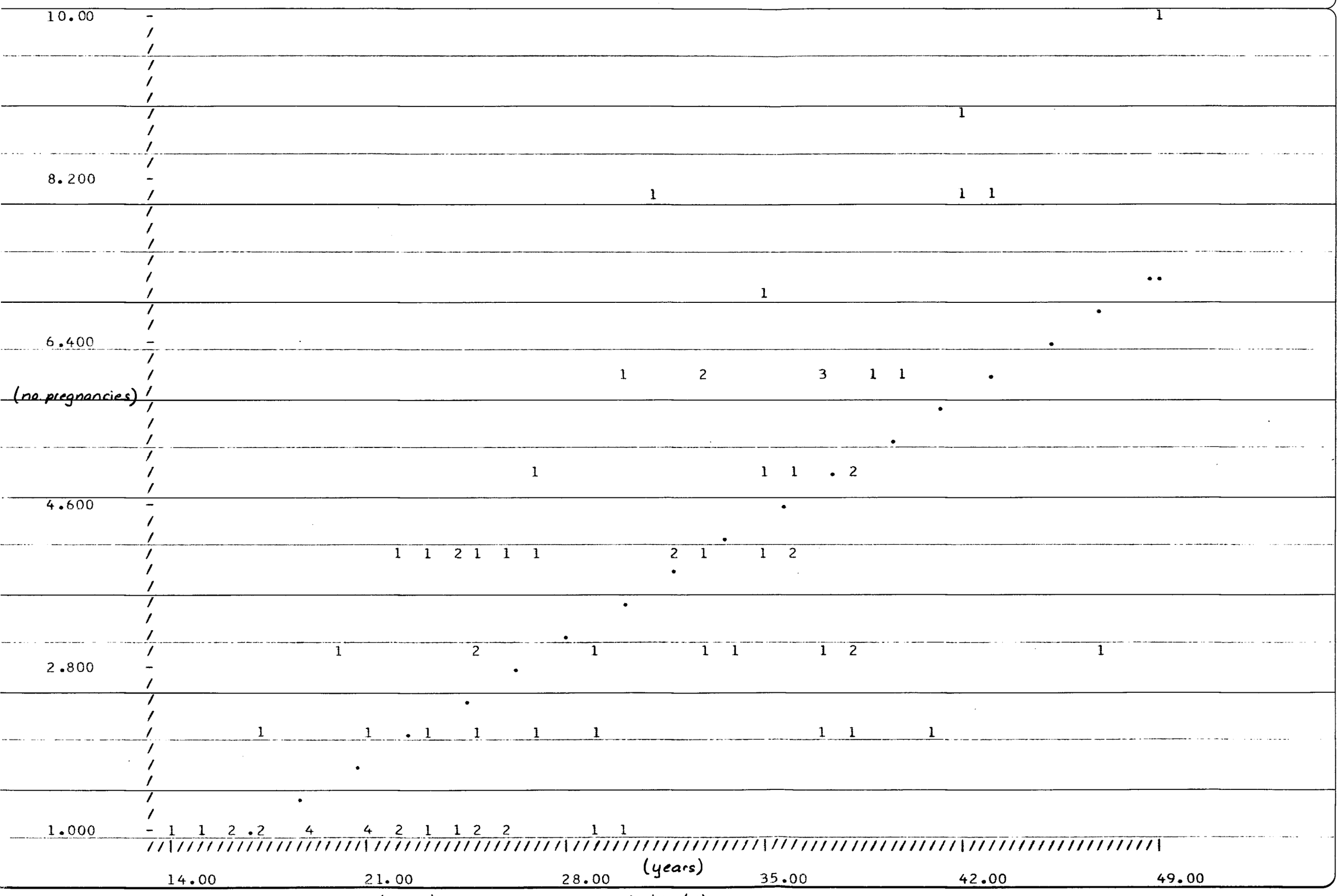

Exhibit 36. Maternal age (years) versus maternal gravidity (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
M-GRA	M-PAR	1.406	1.011	215.4	0.0000	0.1874	0.6889D-01	1.038	0.7937

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS
"A" REPRESENTS 10 OR MORE DATA POINTS

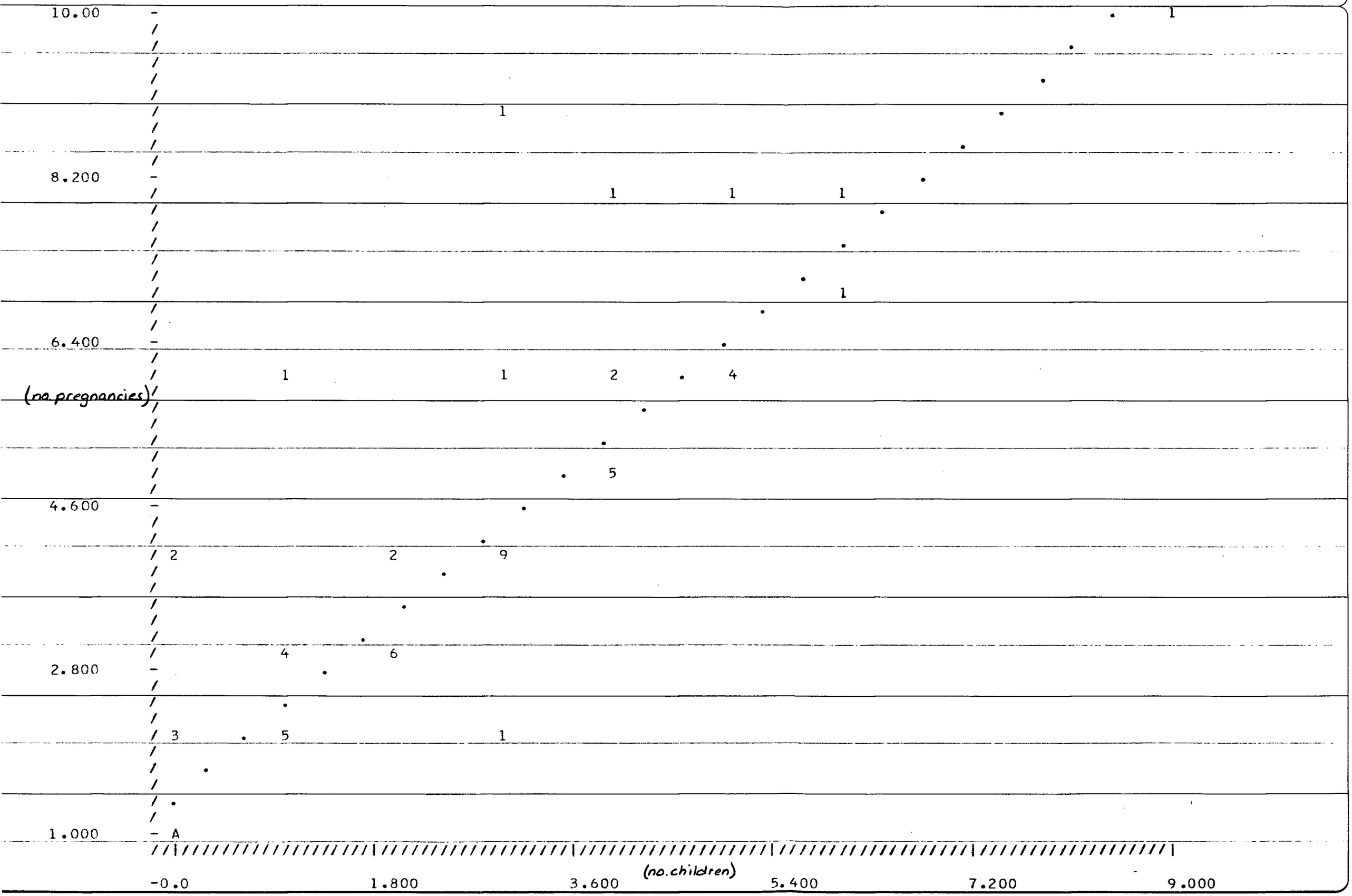

Exhibit 37. Maternal parity (x) versus maternal gravidity (y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
SESGP	SESSC	0.9613	0.5070D-01	202.2	0.0000	0.1274	0.3566D-02	0.3467	0.7831

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

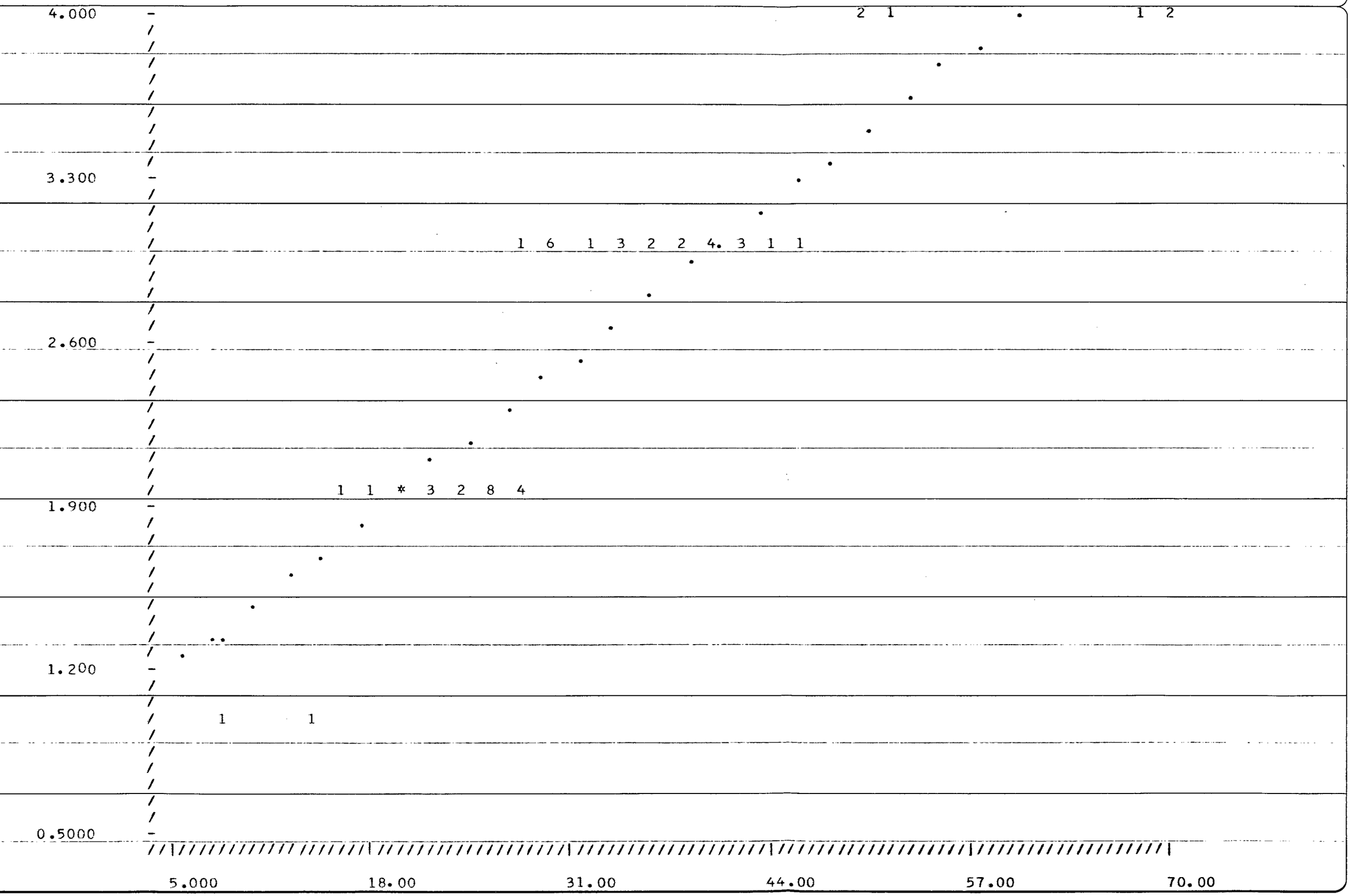

Exhibit 38. Socio-economic score (X) versus socio-economic group (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
N-WSC	N-TOT	6.160	0.1670	342.0	0.0	0.8297	0.9027D-02	1.364	0.8593

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

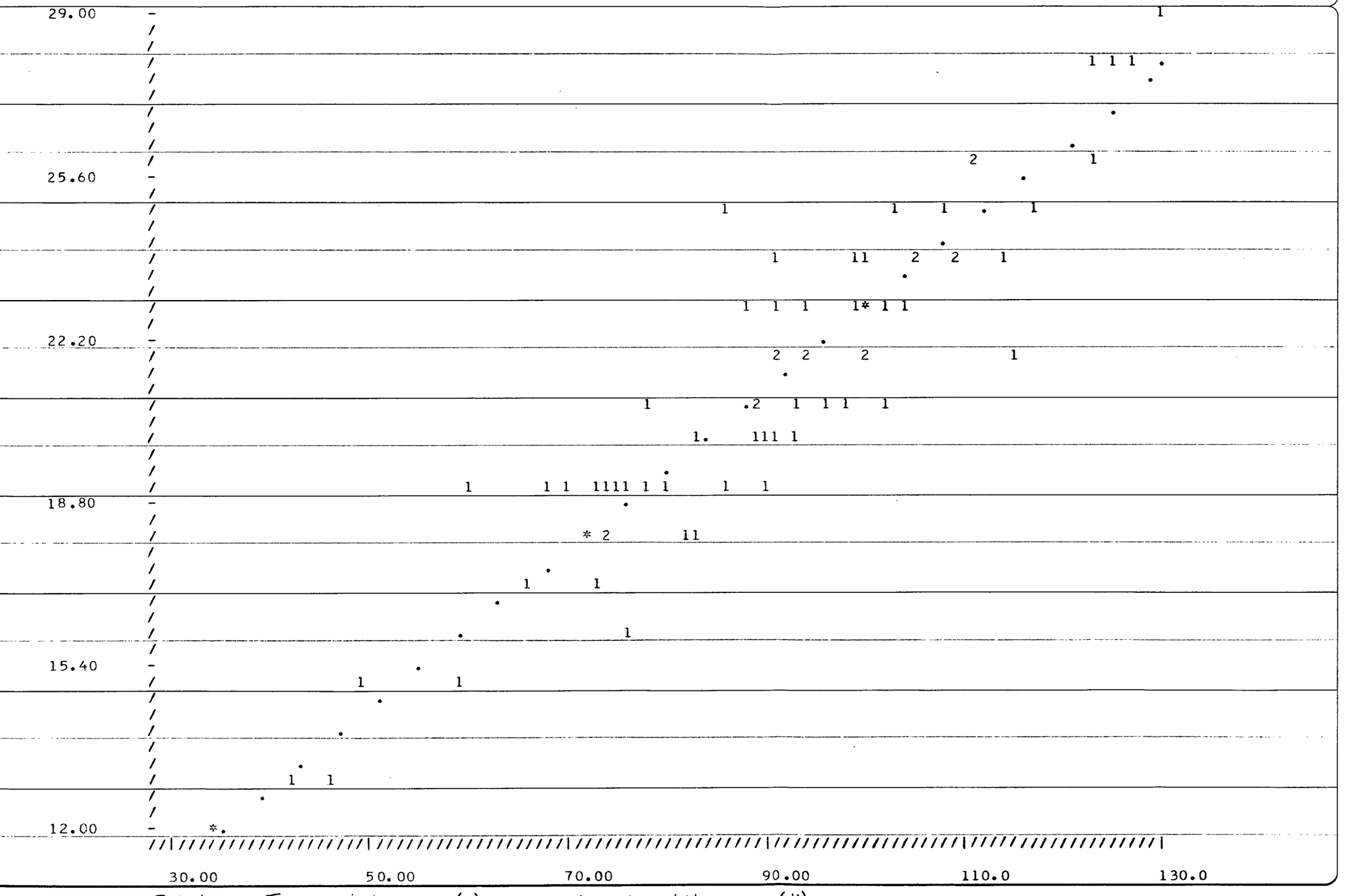

Exhibit 39. Total nutrition score (X) versus weighted nutrition score (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
N-IDX	N-TOT	1.050	0.2762D-01	328.5	0.0	0.1401	0.1524D-02	0.2303	0.8544

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

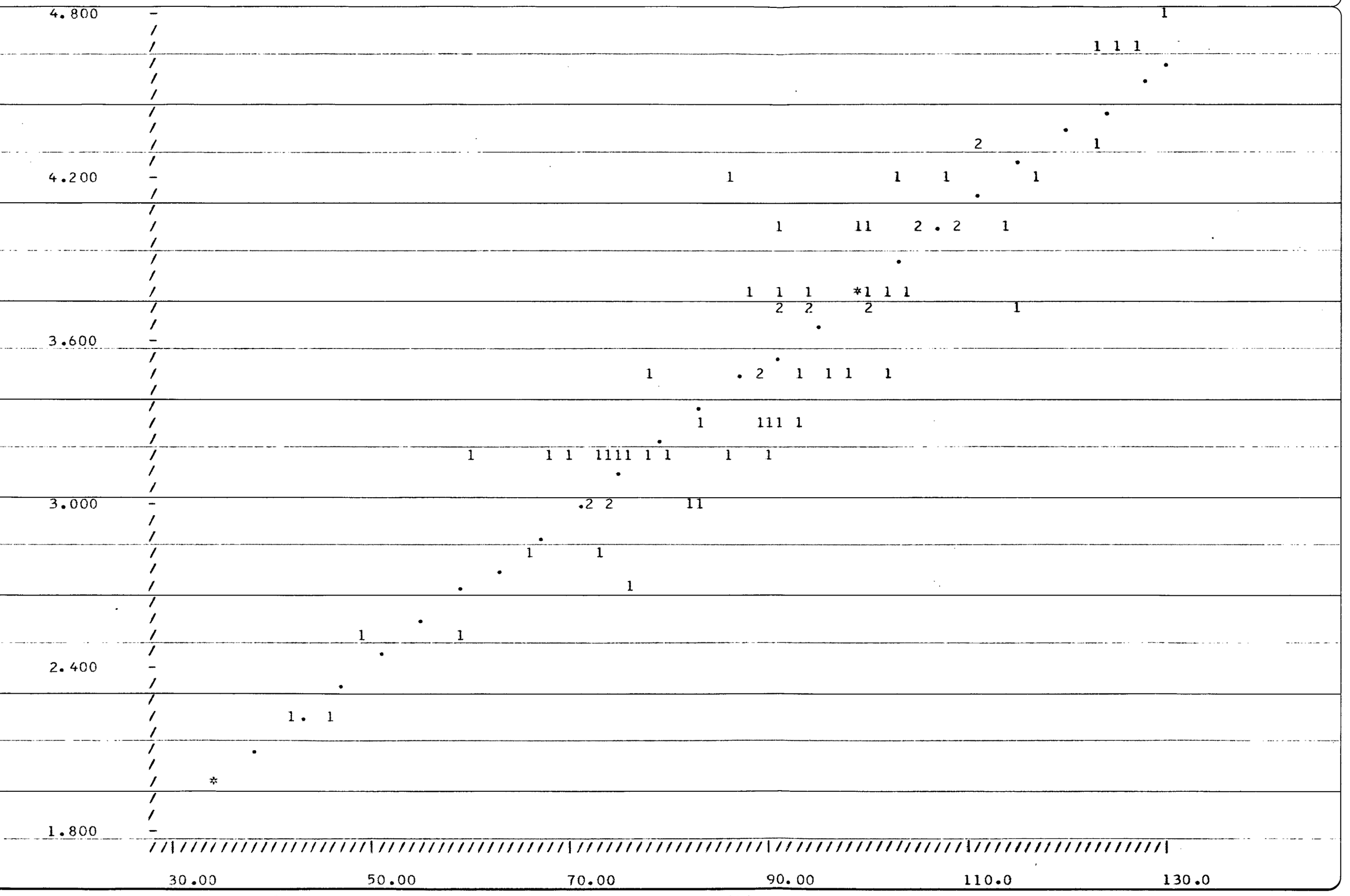

Exhibit 40. Total nutrition score (X) versus nutrition index (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
N-IDX	N-WSC	0.2448E-01	0.1657	0.2469D 05	0.0	0.2262D-01	0.1055D-02	0.2871D-01	0.9977

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS
"A" REPRESENTS 10 OR MORE DATA POINTS

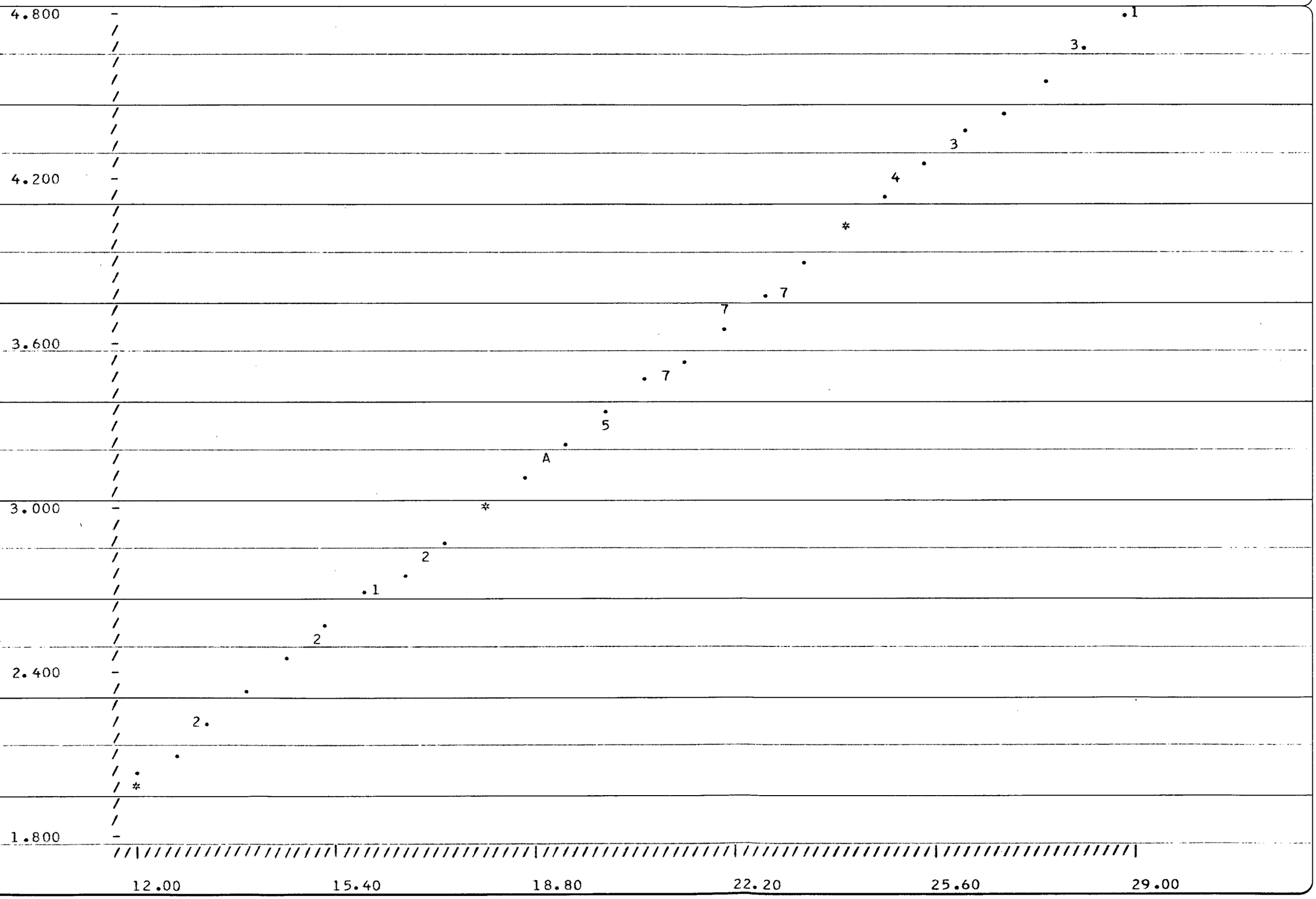

Exhibit 41. Nutrition index (Y) versus weighted nutrition score (X)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
N-PRO	N-TOT	-7.749	0.3947	43.59	0.0000	5.495	0.5978D-01	9.033	0.4377

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

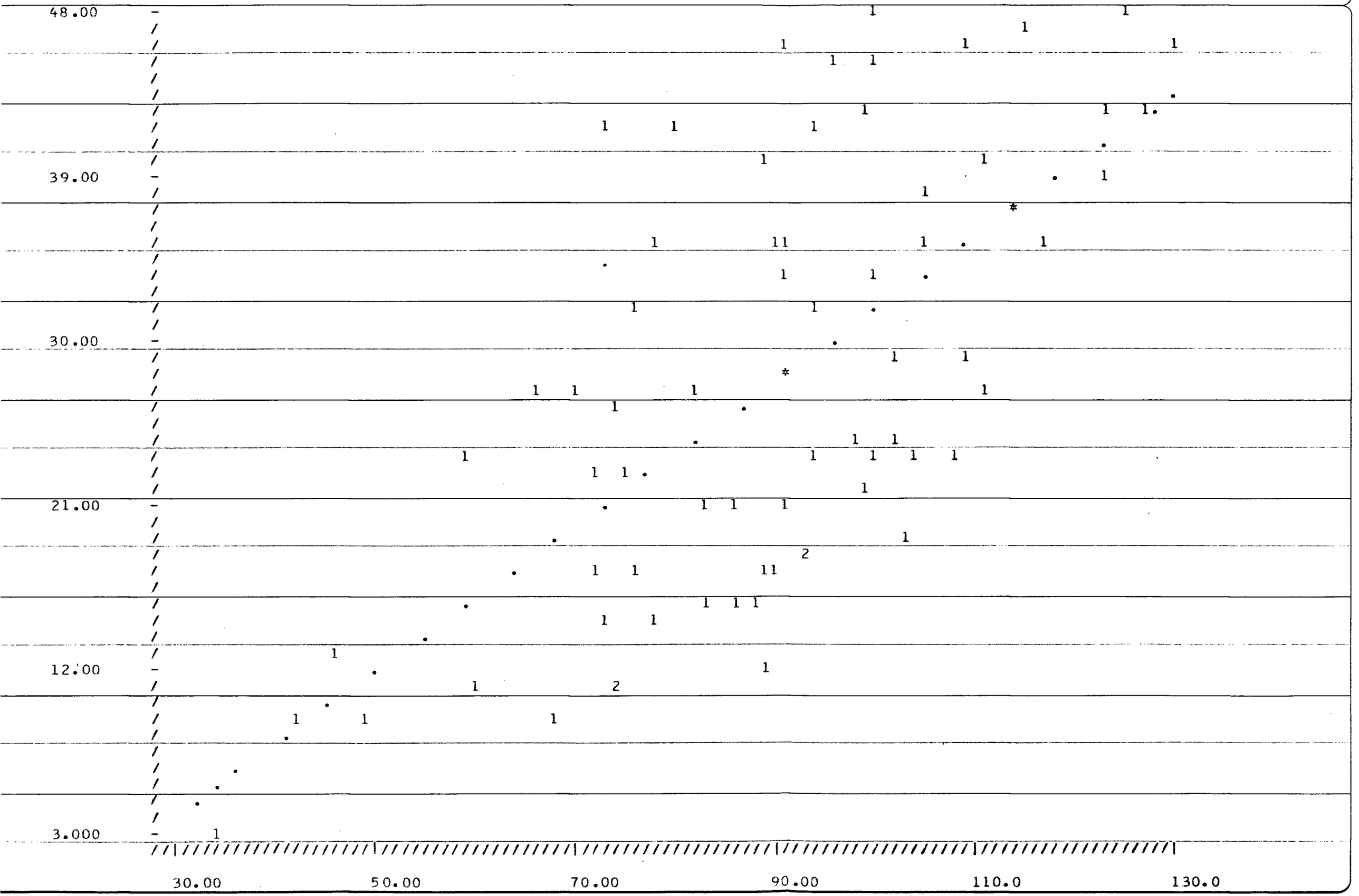

Exhibit 4a. Total nutrition score (X) versus protein score (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
N-WSC	N-PRO	16.39	0.1718	26.83	0.0000	0.9983	0.3317D-01	2.990	0.3239

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

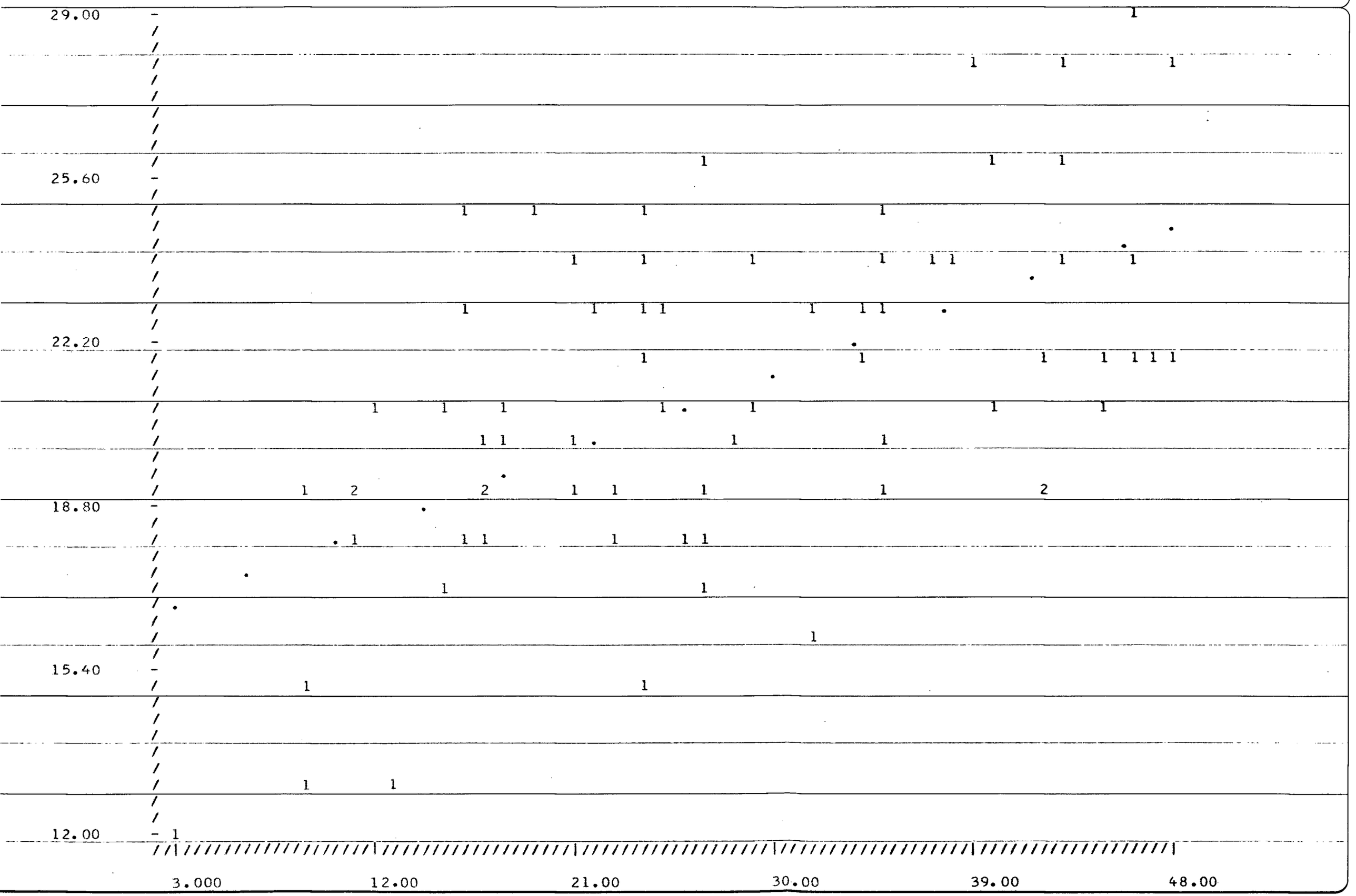

Exhibit 43. Protein score (X) versus weighted nutrition score (Y)

DEP VAR	IND VAR	CONST A	COEFF B	FRATIO (B)	FPROB (B)	STD ERR (A)	STD ERR (B)	STD ERR (Y)	RSQ
N-IDX	N-PRO	2.733	0.2876D-01	27.54	0.0000	0.1649	0.5480D-02	0.4941	0.3296

THE "." AND "*" ARE USED TO PLOT THE REGRESSION LINE; THE "*" IS USED WHEN A PLOT POINT COVERS DATA POINTS

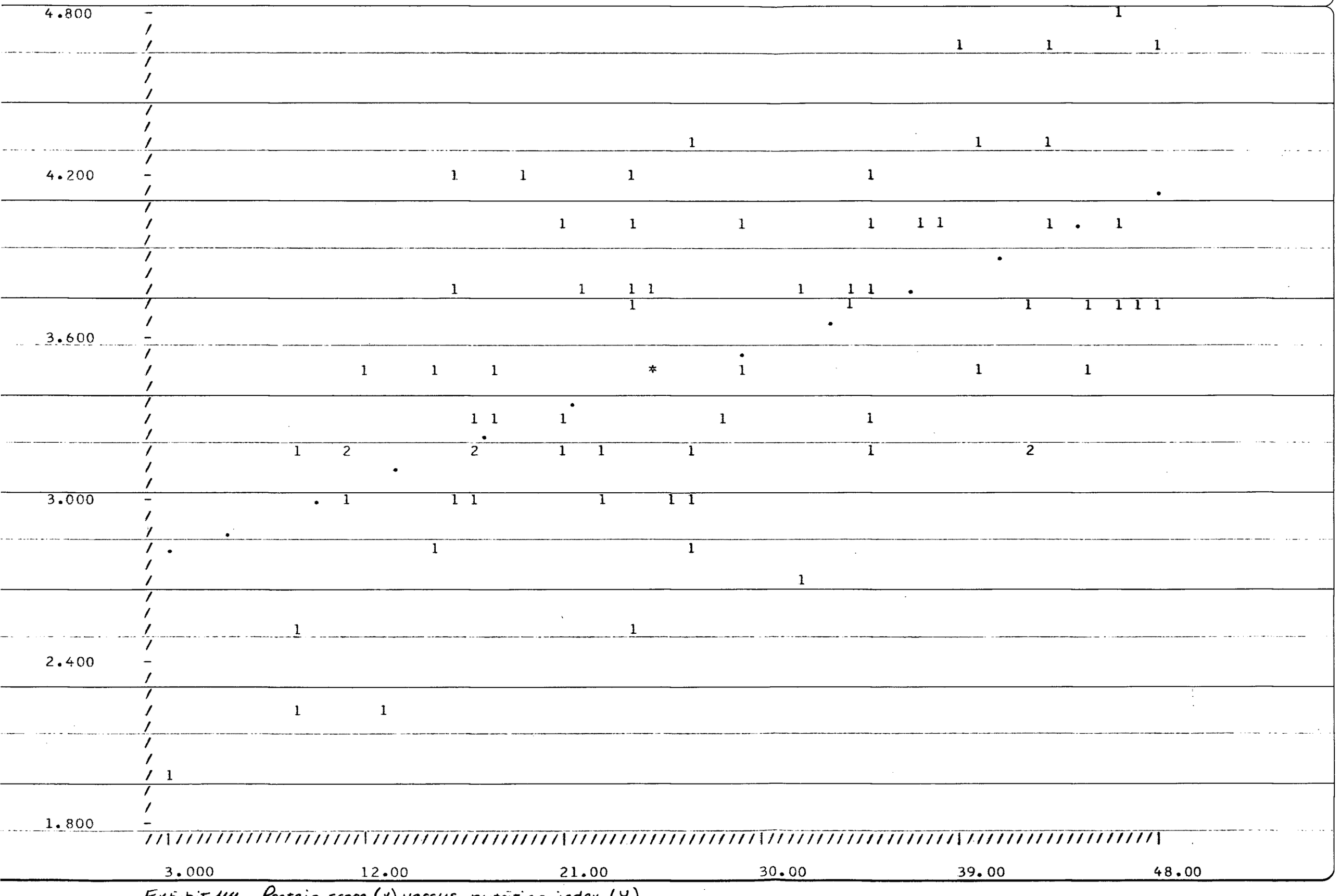

Exhibit 44. Protein score (x) versus nutrition index (Y)