ROY M. ACHESON*

Department of Epidemiology and Public Health, Yale University School of Medicine

A HYPOTHESIS REGARDING THE POSSIBLE ROLE OF ANABOLIC STEROID HORMONES AS PROMOTORS OF NEOPLASIA OF BONE AND BONE MARROW AT ADOLESCENCE

In 1958 Price[1] made an interesting comparison between normal and pathological growth when he demonstrated that the curves of relative incidence of certain bone tumors plotted against age bear a strong resemblance to curves showing the normal growth rate of children. "There can be little doubt," he concluded, "that the relatively earlier age at which both bone forming tumours (i.e. osteogenic sarcoma and osteochondroma) appear in girls as compared with boys is due to the advanced bone age of girls."

Three years later Lee[2] drew attention to the fact that in many countries leukemia mortality figures show a small peak between the tenth and twentieth year in both sexes. Although the precise timing varies a little between one country and another, in each of the four he studied the age of maximum mortality is earlier for girls than for boys, and the increase in death rates seems to be chiefly due to acute myeloid leukemia. Neither Lee himself nor Court Brown and Doll,[3] who confirmed Lee's observation in a simultaneously published paper, hazard any guesses as to the pathogenesis of acute myeloid leukemia of adolescence, although Lee does draw attention to the fact that it occurs at a time of rapid bone growth. It is the purpose of this paper to present evidence which indicates that a factor promoting the neoplasia in these bone and bone marrow neoplasms may be the increased output of anabolic steroid hormones which precedes puberty.

PHYSIOLOGICAL CELL RESPONSES AT PUBERTY

The pioneer observations of J. C. Aub, I. T. Nathanson and their colleagues[4,5] based on repeated urine assays over a period of time on a group of boys and girls have shown that the pre-pubertal growth spurt is preceded in both sexes by a gradually increasing output of both androgenic and estrogenic hormones. Furthermore, just as the growth spurt itself is earlier in girls than in boys, so does the rise in production of sex hormones begin

* Associate Professor of Epidemiology and Medicine.
Received for publication 15 April 1963.

earlier in females.[4-7] Subsequent workers have accepted the fact that these hormones are actually the *cause* of the considerable increase in growth rate during puberty and have gone so far as to call these final developmental changes "the steroid phase of growth."[8-11] Growth in stature is almost wholly due to the performance of the chondroblasts in the germinal layer of the growth cartilage plates of the long bones of the legs, and of the vertebrae; minor contributions are made by the skull and by sub-periosteal activity elsewhere but the latter amounts to a fraction of one per cent

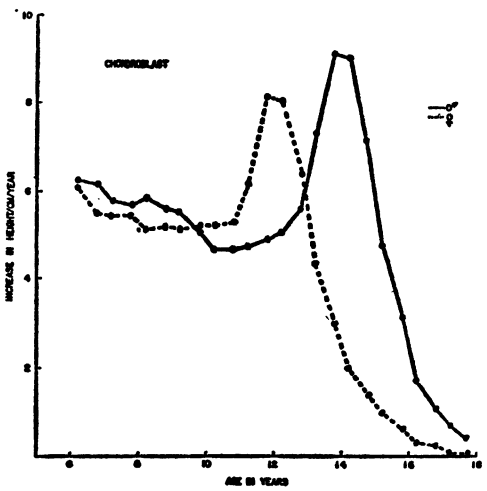

FIG. 1. The annual growth in stature in centimetres per year for boys and girls. These curves are primarily a reflection of the activity of chondroblasts in the growth cartilage plates of the bones of the lower limbs and of the spine (after Tanner[11]).

of all growth. Thus the increase in rate of growth shown in Figure 1, which on average reaches its peak in girls aged 12 years and boys aged 14 years, can be taken as an indication that the mitosis of normal chondroblasts is accelerated by the anabolic steroid hormones. The chondroblast is, of course, only one of the skeletal cells derived from the embryonic mesoblast, but as Figures 2 and 3 show, both the osteoblast*[12] and, at least in the special circumstances concerned with the growth of the pituitary fossa, the osteoclast[14] show increased mitotic activity during the pre-

*It is not relevant to burden the text of this paper with details of how osteoblastic or osteoclastic activity were measured and releated to puberty. Readers interested in these particulars are advised to refer to the original papers.[12-14]

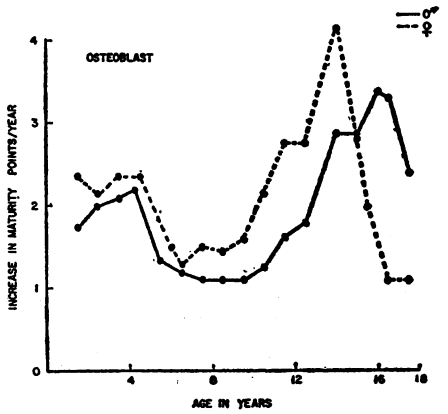

FIG. 2. The annual increase in the maturity of the hip joint and pelvis per year; there is a peak in females in the 14th and in males in the 16th year. Skeletal maturation was assessed by studying the ossification pattern of healthy children from serial X-rays, and is a measure of osteoblastic activity (after Hewitt and Acheson[13]).

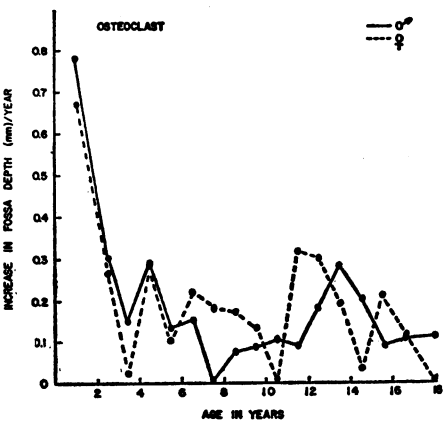

FIG. 3. The annual increase in the depth of the pituitary fossa, measured from serial X-rays of healthy children. Note that the peak growth rate in pubertal girls is in the 12th year and in pubertal boys in the 14th year; it was concluded that this deepening of the fossa was caused by the activity of osteoclasts (after Acheson and Archer[14]).

pubertal growth spurt, and it has been concluded that this increase in rate is also stimulated by the anabolic steroid hormones.[13] Note that for these cells, too, the peak rate for females precedes that for males.

The other important derivatives of the embryonic mesoblast within the fabric of the skeleton itself are the reticulum or stem cells responsible for

hemopoiesis. I know of no longitudinal data on blood formation in man similar to the studies reviewed on bone forming cells; moreover counts of blood cells of any kind are liable to considerable physiological variation and experimental error. Added to this, increments calculated from cross-sectional data carry with them very wide confidence limits.[15] With such reservations borne in mind it is nevertheless of interest that both for

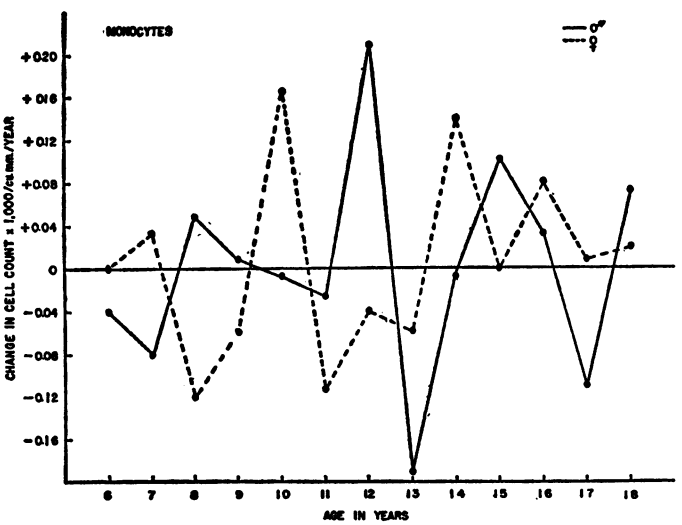

FIG. 4. Annual changes in the monocyte count per year. Unlike the data presented in Figure 1, these curves were calculated from purely cross-sectional material and are therefore liable to a considerable sampling error. There is, however, a suggestion, albeit inconclusive, of a female peak in the 10th year, followed by a male peak in the 12th year. (Calculated from data of Osgood, *et al.*[15-17]).

monocyte production,[16-18] and for hemoglobin formation[19] there is reason to believe that a period of accelerated production in the female is followed by a similar acceleration in the male (see Figs. 4 and 5). Not surprisingly, however, similar data failed to show any such peak for the more variable polymorphonuclear cell counts. Yet this paucity of longitudinal epidemiological data to support the present hypothesis is offset by work in the cellular fields. Starting with the pioneer investigations of Pfeiffer and Gardner,[20] evidence from classical histological studies[21, 22] has been accumulating to the effect that "osteoblasts, osteocytes, osteoclasts and reticular cells are the same cell type."[23] Young[24] has recently confirmed these conclusions using H^3 thymidine as a tracer in autoradiographic investigations of DNA metabolism in the young rat. Thus it would seem that not only

do bone and bone marrow arise from the same embryonic tissue, the mesoblast, but that in birds and young animals (and presumably children, too) "the various types of bone cell represent different functional states of the same cell"[24]—a precursor to bone and bone marrow which maintains the power of metamorphosis long after the embryonic stage is past.

Therefore in the purely physiological circumstance of normal growth, while mitosis is grossly accelerated in chondroblasts in response to anabolic

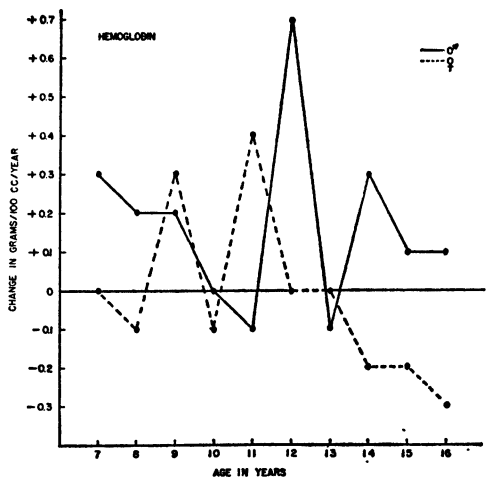

FIG. 5. Changes in hemoglobin content of the blood. A peak in the females in the 11th year is followed by one in males in the 12th year. (Calculated from the cross-sectional data of Hawkins, *et al.*[19]).

steroid hormones, it would seem that acceleration of mitosis is also occurring in other growing cells which are derived from the mesoblast. These may be concerned with either the formation of bone itself or with that of blood inside the bone marrow.

PATHOLOGICAL CELL RESPONSES AT PUBERTY

The curves which Price[1,25] used to show the age-incidence of pathological growth, both of the cartilage cells themselves and of the osteoblasts, are reproduced in Fig. 6. It has subsequently been shown by Mackenzie[26] and his colleagues that the death rate from chondrosarcoma also undergoes an increase at adolescence. The same workers find that at ages 15-19 the male:female ratio of death rates is 2.1:1 and conclude "it is reasonable to relate [this peak mortality] to the adolescent growth spurt." If, as has

been argued above, the anabolic steroid hormones are promotors of accelerated mitosis in all normally growing skeletal tissue, it would seem quite possible that they are also the stimulus which sets off pathological, or neoplastic growth in a cell or cells which may already have in their genetic make-up pre-malignant changes. The mortality rates, by age, for acute myeloid leukemia calculated by Lee[9] show the same general pattern of a female peak followed by a male peak (see Fig. 7); the same arguments

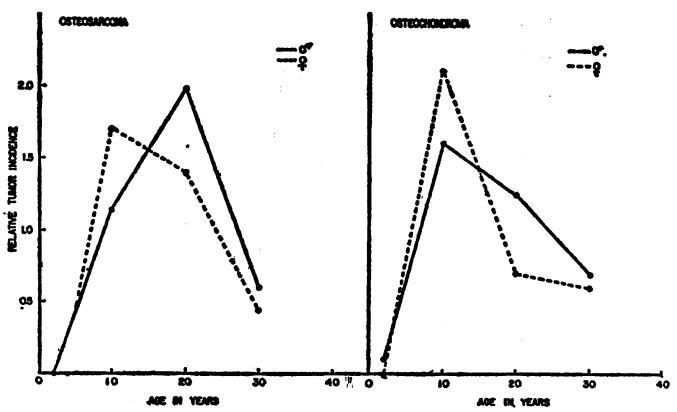

FIG. 6. Relative tumor incidence of osteosarcoma and osteochondroma. In each case there is a peak in the pubertal or immediate post adolescent period, and in each case the curve for the male is to the right of that for the female. (British data, redrawn from Price[1]).

are applicable in relating leukemia to the anabolic steroid hormones as have just been adduced for chondroblastoma and osteosarcoma.

DISCUSSION

It has been suggested that some anabolic steroids, in particular testosterone, may be carcinogens[27-29] and although there is now evidence of this in the case of mammary tissue,[30,31] it is not necessarily true of the neoplasms of the mesenchyme-derived structures which are under consideration here. It is widely held that there are several stages in the metamorphosis of the cell from a normal or a dormant state to one of active neoplasia. In some of the malignant conditions under consideration in this paper chromosome aberrations,[32-37] which may perhaps either be inherited[38-40] or originate from irradiation,[41-46] seem to have a place in the pathogenic train of events, and it has been suggested that viruses may also play a

part in distorting the genetic make up of the cell.[47-49] The epidemiological and other data set out here imply that active neoplasia in such pre-cancerous cells is ignited by normal endocrine secretions; in short, that the skeletal and hemopoietic neoplasms of adolescence are a pathological response to a physical stimulus. An epidemiological method for testing this hypothesis would be to study the lives of those who have died of adolescent cancer with a view to establishing when puberty occurred. Just

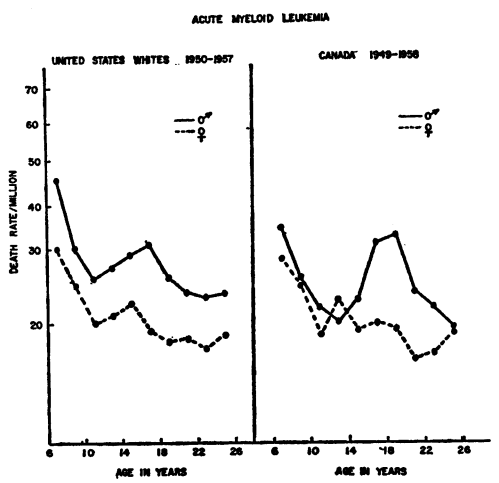

FIG. 7. Death rates for acute myeloid leukemia for males and females. The U.S. curves are smoother than those for Canada because the population at risk is larger and the sampling error proportionately smaller. In both countries a peak is manifest for each sex during the adolescent period and in both countries this is earlier in the female than in the male (redrawn from Lee[2]).

as there is a difference in age of maximum death rate between the sexes, so there should be one *within* each sex; those who came to puberty early would be expected to show an earlier peak death rate than those who developed more slowly.

SUMMARY

Epidemiological and laboratory studies of normal human growth and of malignant conditions of bone and bone marrow are reviewed. As a result, the hypothesis is put forward that the skeletal and hemopoietic neoplasms of adolescence are a pathological response to the physiological stimulus of the anabolic steroid hormones of puberty.

ADDENDUM

Since this paper was submitted I have been able to obtain unpublished information from two longitudinal growth studies[50, 51] which suggest that there is a very well defined pre-pubertal spurt in erythrocyte formation in the male, but no such spurt occurs in the female. The lack of a definitive acceleration in red-cell production in girls is, however, in no way incompatible with the hypothesis set out above.

ACKNOWLEDGEMENTS

My thanks are due to Miss G. B. Fowler for her help in preparing the data, and to colleagues in the Yale School of Medicine for critical comments.

REFERENCES

1. Price, C. H. G.: Primary bone-forming tumours and their relationship to skeletal growth. *J. Bone Jt Surg.*, 1958, *40B*, 574-593.
2. Lee, J. A. H.: Acute myeloid leukaemia in adolescents. *Brit. med. J.*, 1961, *i*, 988-992.
3. Court Brown, W. M. and Doll, R. F. S.: Leukaemia in childhood and young adult life. *Brit. med. J.*, 1961, *i*, 981-988.
4. Nathanson, I. T., Towne, L. E. and Aub, J. C.: Daily excretion of urinary androgens in normal children. *Endocrinology*, 1939, *24*, 335-338.
5. Nathanson, I. T., Bretney Miller, R., Towne, L. E. and Aub., J. C.: The close correlationship of androgen and creatinine excretion in normal children. *Endocrinology*, 1941, *28*, 866-870.
6. Greulich, W. W., Dorfman, R. I., Catchpole, H. R., Solomon, C. I. and Cullotta, C. S.: Somatic and endocrine studies of puberal boys. *Monogr. Soc. Res. Child. Develop.*, 1942, *7*, No. 3.
7. Hamburger, C., Halversen, K. and Pedersen, J.: Assay of androgenic substances in the urine of normal men and women. *Acta pharmacol. (Kbh.)*, 1945, *1*, 129-140.
8. Kinsell, L. W., Michaels, G. D., Li, C. H., and Larsen, W. E.: Studies in growth. I. Interrelationship between pituitary growth factor and growth promoting androgens in acromegaly and gigantism. *J. clin. Endocr.*, 1948, *8*, 1013-1036.
9. Garn, S. M.: Physical growth and development. *Amer. J. phys. Anthropol.*, 1952, *10*, 169-192.
10. Hewitt, D. and Acheson, R. M.: Some aspects of skeletal development through adolescence. II. The interrelationship between skeletal maturation and growth at puberty. *Amer. J. phys. Anthropol.*, 1961, *19*, 333-334.
11. Tanner, J. M.: *Growth at Adolescence*, 2nd ed. Oxford, Blackwell, 1962.
12. Hewitt, D. and Acheson, R. M.: Some aspects of skeletal development through adolescence. I. Variations in the rate of pattern of skeletal maturation at puberty. *Amer. J. phys. Anthropol.*, 1961, *19*, 321-332.
13. Acheson, R. M.: The Oxford method of assessing skeletal maturity. *Clin. Orthop.*, 1957, *10*, 19-39.
14. Acheson, R. M. and Archer, M.: Radiological studies of the growth of the pituitary fossa in man. *J. Anat. (Lond.)*, 1959, *93*, 52-67.
15. Tanner, J. M.: Some notes on the reporting of growth data. *Hum. Biol.*, 1951, *23*, 93-159.
16. Osgood, E. E., Barber, R. L., Brownlee, I. E., Osgood, M. W., Ellis, D. M. and Cohen, W.: Total, differential and absolute leukocyte counts and sedi-

mentation rates of healthy children 4-7 years of age. *Amer. J. Dis. Child.*, 1939, *58*, 61-70.

17. Osgood, E. E., Barber, R. L., Brownlee, I. E., Osgood, M. W., Ellis, D. M. and Cohen, W.: Total, differential and absolute leukocyte counts and sedimentation rates of healthy children, standards for 8 to 14 years of age. *Amer. J. Dis. Child.*, 1939, *58*, 282-294.
18. Osgood, E. E., Barber, R. L., Brownlee, I. E., Osgood, M. W., Ellis, D. M. and Cohen, W.: Total, differential and absolute leukocyte counts and sedimentation rates of healthy adolescents 15 to 18 years of age. *J. Lab. clin. Med.*, 1938, *24*, 905-912.
19. Hawkins, W. W., Speck, E., and Leonard, V. G.: Variation in the hemoglobin level with age and sex. *Blood*, 1954, *9*, 999-1007.
20. Pfeiffer, C. A. and Gardner, W. U.: Skeletal changes and blood serum calcium level in pigeons receiving estrogens. *Endocrinology*, 1938, *23*, 485-491.
21. Bloom, W., Bloom, M. A. and McLean, F. C.: Calcification and ossification. Medullary changes in the reproductive cycle of female pigeons. *Anat. Rec.*, 1941, *81*, 445-500.
22. Prichard, J. J.: The osteoblast. In, *The Biochemistry and Physiology of Bone*. Bourne, G. H. (Ed.) New York, Academic Press, 1956, chapter 7, pp. 179-212.
23. Bloom, M. A., Domm, L. V., Nalbandov, A. V. and Bloom, W.: Medullary bone of laying chickens. *Amer. J. Anat.*, 1958, *102*, 411-454.
24. Young, R. W.: Cell proliferation and specialization during endochondral osteogenesis in young rats. *J. Cell Biol.*, 1962, *14*, 357-370.
25. Price, C. H. G.: Osteogenic sarcoma: an analysis of the age and sex incidence. *Brit. J. Cancer*, 1955, *9*, 559-574.
26. Mackenzie, A., Court Brown, W. M., Doll, R., and Sissons, H. A.: Mortality from primary tumours of bone in England and Wales. *Brit. med. J.*, 1961, *i*, 1782-1790.
27. Aub, J. C. and Nathanson, I. T.: Neoplastic diseases. *Ann. Rev. Med.*, 1951, *2*, 343-366.
28. Wislocki, G. B., Aub, J. C. and Waldo, C. M.: Effects of gonadectomy and the administration of testosterone propionate on the growth of antlers in male and female deer. *Endocrinology*, 1947, *40*, 202-224.
29. Aub, J. C., Wislocki, G. B., and Waldo, C. M.: The growth of the deer antler; how it may lead to a better understanding of cancer. *N.Y. State Conservationist*. 1950, Reprint No. 98.
30. Bulbrook, R. D., Hayward, J. L. Spicer, C. C., and Thomas, B. S.: Comparison between urinary steroid excretion of normal breast cancer and women with advanced breast cancer. *Lancet*, 1962, *ii*, 1235-1237.
31. Bulbrook, R. D., Hayward, J. L., Spicer, C. L. and Thomas, B. S.: Abnormal excretion of urinary steroids by women with early breast cancer. *Lancet*, 1962, *ii*, 1238-1240.
32. Nowell, P. C. and Hungerford, D. A.: A minute chromosome in human chronic granulocytic leukemia. *Science*, 1960, *132*, 1497.
33. Baikie, A. G., Court Brown, W. M., Jacobs, P. A., and Milne, J. S.: Chromosome studies in common leukaemia. *Lancet*, 1959, *ii*, 425-428.
34. Tough, I. M., Court Brown, W. M., Baikie, A. G., Buckton, K. E., Harnden, D. G., Jacobs, P. A., King, M. J., and McBride, J. A.: Cytogenetic studies in chronic myeloid leukaemia and acute leukaemia associated with mongolism. *Lancet*, 1961, *i*, 411-417.
35. Spriggs, A. I., Boddington, M. M., and Clarke, C. M.: Chromosomes of human cancer cells. *Brit. med. J.*, 1962, *ii*, 1431-1435.
36. Hungerford, D. A. and Nowell, P. C.: Chromosomal studies in human leukemia. III. Acute granulocytic leukemia. *J. nat. Cancer Inst.*, 1962, *29*, 545-565.
37. Weinstein, A. W. and Weinstein, E. D.: A chromosomal abnormality in acute myeloblastic leukemia. *New Engl. J. Med.*, 1963, *268*, 253-254.

38. Stewart, A. M.: Aetiology of childhood malignancies. *Brit. med. J.*, 1961, *i*, 452-460.
39. Buckton, K. E., Harnden, D. G., Baikie, A. G., and Woods, G. E.: Mongolism and leukaemia in the same sibship. *Lancet*, 1961, *i*, 171-172.
40. Baikie, A. G., Buckton, K. E., Court Brown, W. M., and Harnden, D. G.: Two cases of leukaemia. *Lancet*, 1961, *ii*, 1003-1004.
41. Stewart, A. M., Webb, J., and Hewitt, D.: A survey of childhood malignancies. *Brit. med. J.*, 1958, *i*, 1495-1508.
42. Armitage, P., Court Brown, W. M., and Doll, R.: Dose-response relationship in radiation leukaemia. *Nature*, 1959, *184*, 1669-1670.
43. MacMahon, B.: Prenatal X-ray exposure and childhood cancer. *J. nat. Cancer Inst.*, 1962, *28*, 1173-1191.
44. *Lancet* editorial: Harmful effects of diagnostic radiation. *Lancet*, 1963, *i*, 255-256.
45. Aub, J. C., Evans, R. D., Hempelmann, L. H., and Martland, H. S.: Late effects of internally-deposited radioactive materials in man. *Medicine*, 1952, *31*, 221-329.
46. Looney, W. B.: Late skeletal roentgenographic histopathological, autoradiographic and radiochemical findings following radium deposition. *Amer. J. Roentgenol.*, 1956, *75*, 559-572.
47. Smadel, J. E.: Tumor virus research program. In *Symposium on Phenomena of the Tumor Viruses*, Beard, J. W., Ed. *Nat. Cancer Inst. Monogr.*, 1960, No. 4, pp. 1-5.
48. Moloney, J. B.: Properties of a leukemia virus. In *Symposium on Phenomena of the Tumor Viruses*, Beard, J. W., Ed. *Nat. Cancer Inst. Monogr.*, 1960, No. 4, pp. 7-37.
49. Syverton, J. T.: Present status of studies on tumor producing viruses. In *Cancer Symposium*, Leister, J. W., Ed. *Nat. Cancer Inst. Monogr.*, 1960, No. 4, pp. 345-353.
50. Bouterline-Young H.: Florence-Harvard Longitudinal Growth Studies (personal communication).
51. Meyers A. and McCammon R.: Child Research Council, Denver Longitudinal Growth Studies (personal communication).