Concentration of Chromium in the Hair of Normal Children and Children with Juvenile Diabetes Mellitus

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SUMMARY

The concentration of chromium in the hair of thirty-three normal children and nineteen children with juvenile-onset diabetes mellitus was measured by atomic absorption spectrophotometry. The geometric mean for the group of normal children was 0.85 μg./gm.; range (mean ± 2 SD) 0.36 — 1.87 μg./gm., and for the diabetic children, 0.56 μg./gm. (range, 0.26 — 1.19 μg./gm.). Comparison of these results revealed a highly significant difference ($p < 0.001$) between the two groups. DIABETES 17:517-19, August, 1968.

A relationship between chromium deficiency and impaired glucose tolerance has been established in laboratory animals,[1-4] in adult man[5-7] and possibly in children.[8] The pattern of plasma chromium response to an oral glucose load provides one means of detecting chromium deficiency.[9] The measurement of plasma chromium levels still presents considerable technical difficulty, however, and the requirement for relatively large samples is a major problem in the pediatric age group.

Analysis of hair has been successfully employed as a means of detecting both deficiency[10] and excess[11] of trace elements in the body. A characteristic of many trace elements including chromium[12,13] is that, normally, they are concentrated in hair at least ten times that of ambient blood levels. The problem of analytical sensitivity is therefore obviated. The present study was undertaken to establish the range of hair chromium concentration in normal children and to compare it with values obtained on the hair of children with juvenile diabetes mellitus.

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MATERIAL AND METHODS

Hair samples weighing approximately 1 gm. were obtained from children with juvenile diabetes, the majority of whom had been receiving insulin for several years. Control samples were collected from apparently healthy nonrelated children living in the same area. No particular hair length or site was chosen for sampling, but it was believed that the size of the sample probably negated any variation between individual hairs.[12] The samples were washed sequentially in redistilled hexane, analytical grade ethyl alcohol and finally three times with deionized water. The hair was then dried overnight in an oven at 110°, allowed to cool and weighed.

The dried hair samples were digested in 13.5 ml. of a mixture of redistilled acids consisting of nitric acid, perchloric acid and sulfuric acid in the ratio of 10:2.5:1, until a colorless solution was obtained. The digest was diluted with deionized water and the chromium present oxidized to the hexavalent state by heat and the addition of potassium permanganate. Extraction of the chromium from this aqueous solution was achieved by the use of 2 ml. of methyl-iso-butyl ketone.[14]

The concentration of chromium in the organic extract was determined by atomic absorption spectrophotometry with a Jarrell-Ash model 82-362 single beam atomic absorption spectrophotometer equipped with a Sargent model SR variable range recorder and a Disc model 204 chart integrator. The atomic absorption spectrophotometer was adapted for scale expansion,[15] and results were calculated on the basis of the integrals of the recorded curves generated by a measured volume of organic extract.[16]

Standard chromium solutions in methyl-iso-butyl ketone were prepared from potassium dichromate covering

a concentration range of 0.125 to 1.0 μg./ml.

The coefficient of variation (CV) of standard chromium solutions (range 0.125 — 1.0 μg./ml.) was 6.9 per cent (ninety one samples). Mean recovery from the organic extract of trivalent chromium-51 added to twenty-one hair samples prior to ashing was 103.6 per cent (CV 7.8 per cent).

RESULTS

The individual results for thirty-three normal children and nineteen children with juvenile diabetes are shown in table 1.

Both groups gave a skewed distribution; however, following a logarithmic transformation of the results, probit plots indicated that the distributions had been normalized.[17,18] In addition, the transformation proved to be variance stabilizing and Snedecor's *F* test gave acceptable evidence of the homogeneity of the variances.

The geometric mean for the thirty-three normal children was found to be 0.85 μg./gm. with a range (mean ± 2 SD) of 0.36 — 1.87 μg./gm. The regression on age was not significant and no significant difference was found between the values by sex. For the nineteen children with juvenile diabetes, the geometric mean was 0.56 μg./gm. and the range 0.26 — 1.19 μg./gm. There was no relation to age or sex. Comparison of the mean values of the two groups with Student's t test revealed a highly significant difference ($p < 0.001$).

DISCUSSION

Variation in the concentration of chromium in hair is detectable only over a period of many weeks.[19] While this eliminates the potential usefulness of this assay as a means of monitoring short-term changes in chromium states, it may in other circumstances be advantageous. For example, this measurement, in contrast to that of plasma chromium, would not be subject to temporary fluctuations due to the influence of factors such as recent glucose intake.

A positive correlation between hair chromium and the concentration of this element in other body tissues awaits final confirmation although by analogy with other transition elements[10,20] this might be expected. With this assumption, two possible explanations for the difference observed between the two groups are suggested.

Firstly, a reduction in the total body content of chromium, possibly resulting from a nutritional deficit, could have preceded the onset of clinical symptoms. It is feasible that such a deficiency could be one environmental factor influencing the precipitation of overt disease in a genetically predetermined juvenile diabetic.

Secondly, depleted levels of chromium in tissue may be secondary to abnormal carbohydrate metabolism. It has been shown for example, that an excessive loss of chromium in the urine occurs following an oral glucose load.[21] This loss, which presumably follows an increase in plasma chromium levels, could be mediated through an increase in plasma insulin[5] and could, therefore, be

TABLE 1

Hair chromium concentration (μg./mg.)

Normal children				Juvenile diabetic children			
Female	Age in years	Male	Age in years	Female	Age in years	Male	Age in years
0.40	12	0.45	7	0.25	7	0.35	11
0.50	4	0.50	4	0.45	4	0.40	3
0.50	12	0.55	4	0.45	4	0.40	15
0.50	11	0.60	1	0.50	5	0.40	13
0.55	6	0.65	6	0.50	6	0.55	15
0.60	2	0.70	1	0.50	15	0.70	8
0.60	10	0.75	6	0.60	6	0.75	7
0.70	8	0.80	3	0.70	10	1.00	10
0.70	13	0.80	3	0.80	11	1.10	10
0.75	5	0.80	3	0.90	13	—	—
0.90	3	0.80	4				
0.90	1	1.10	11				
1.00	1	1.50	2				
1.10	2	1.60	1				
1.10	12	2.00	2				
1.30	12	—	—				
1.40	10	—	—				
1.60	4	—	—				

Geometric mean 0.85 μg./gm.
Range (Mean ± 2 SD) 0.36-1.87 μg./gm.

Geometric mean 0.56 μg./gm.
Range (Mean ± 2 SD) 0.26-1.19 μg./gm.

iatrogenic in the insulin-treated diabetic. It was not possible to determine in the present study if the decreased chromium levels were related to duration of known diabetes in insulin treatment.

Hair chromium concentrations in the adult appear to be influenced both by sex and parity,[22] but the mean levels of all adult groups are lower than those of normal children. This finding lends support to the concept of declining tissue concentration with age.[23] It should be emphasized, however, that relatively low results were obtained from individuals of all groups, indicating that chromium deficiency may not be the sole prerogative of the elderly. In this respect, it is noteworthy that the only sample of hair which contained no detectable chromium was collected from a nulliparous seventeen-year-old girl with juvenile-onset diabetes. In the light of the differences noted in hair chromium, the precise role of this trace element in juvenile diabetes should be further investigated.

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REFERENCES

[1] Schwarz, K., and Mertz, W.: Chromium (III) and the glucose tolerance factor. Arch. Biochem. Biophys. *85*:292, 1959.

[2] Mertz, W., Roginski, E., and Schwarz, K.: Effect of trivalent chromium complexes on glucose uptake by epididymal fat tissues of rats. J. Biol. Chem. *236*:318, 1961.

[3] Mertz, W., and Roginski, E.: The effect of trivalent chromium on galactose entry in rat epididymal fat tissue. J. Biol. Chem. *238*:868, 1963.

[4] Schroeder, H. A.: Diabetic-like serum glucose levels in chromium deficient rats. Life Sciences *4*:2057, 1966.

[5] Glinsmann, W., and Mertz, W.: Effect of trivalent chromium on glucose tolerance. Metabolism *15*:510, 1966.

[6] Levine, R. A., Streeten, D. H. P., and Doisy, R. J.: Effects of oral chromium supplementation on glucose tolerance of elderly human subjects. Metabolism 7:114, 1968.

[7] Mertz, W.: Biological role of chromium. Fed. Proc. *26*:186, 1967.

[8] Hopkins, L. L. Jr., Ransome-Kuti, O., and Majaj, A. S.: Improvement of impaired carbohydrate metabolism by chromium (III) in malnourished infants. Amer. J. Clin. Nut. *21*:203, 1968.

[9] Glinsmann, W., Feldman, F., and Mertz, W.: Plasma chromium after glucose administration. Science *152*:1243.

[10] Strain, W. H., Steadman, L. T., Lankau, C. A., Berliner, W. P., and Pories, W. J.: Analysis of zinc levels in hair for the diagnosis of zinc deficiency in man. J. Lab. Clin. Med. *68*:244, 1966.

[11] Kopito, L., Byers, R. K., and Schwachman, H.: Lead in hair of children with chronic lead poisoning. New Eng. J. Med. *276*:949, 1967.

[12] Perkons, A. K., and Jervis, R. E.: Trace elements in human head hair. J. Forensic Sci. *11*:50, 1966.

[13] Prasad, A. S.: Nutritional metabolic role of zinc. Fed. Proc. *26*:172, 1967.

[14] Feldman, F. J., and Purdy, D. C.: The atomic absorption spectroscopy of chromium. Anal. Chim. Acta *33*:273, 1965.

[15] Rodgerson, D. O., and Helfer, R. E.: Determination of iron in serum or plasma by atomic absorption spectrophotometry. Clin. Chem. *12*:338. 1966.

[16] Rodgerson, D. O., and Helfer, R. E.: Recorder curve integration for the quantitation of trace metals by atomic absorption spectrophotometry. Amer. J. Clin. Path. *46*:63, 1966.

[17] Murphy, E. A.: Evaluation of clinical data. Improvement of efficiency by simple transformation. J. Chron. Dis. *15*:795.

[18] Mustard, J. F., Murphy, E. A., Rowsell, H. C., and Downie, H. G.: Factors influencing thrombus formation in vivo. Amer. J. Med. *33*:621, 1962.

[19] Coleman, R. F., Cripps, F. H. Stimson, A. and Scott, H. D.: The determination of trace elements in human hair by neutron activation and the application to forensic science. Atomic Weapons Res. Establishment Report No. o-86/66, 1967.

[20] Gopolan, C., Reddy, V., and Mohan, V.: Some aspects of copper metabolism in protein calorie malnutrition. J. Pediat. *63*:646, 1963.

[21] Schroeder, H. A.: Cadmium, chromium and cardiovascular disease. Circulation *35*:570, 1967.

[22] Hambidge, K. M., and Rodgerson, D. O.: A comparison of hair chromium levels of nulliparous and parous women. Amer. J. Obstet. and Gynecol. In Press.

[23] Schroeder, H. A., Balassa, J., and Tipton, I.: Abnormal trace metals in man—chromium. J. Chron. Dis. *15*:941, 1962.