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Serum Protein-Bound Iodine Levels in Adolescents

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THIS study was prompted by the observation of Dr. C. K. Rowan-Legg, Medical Officer for the Ottawa Secondary School Board, that a number of high school students referred to him because of lagging scholastic peformance showed improvement when thyroid medication was tried empirically. It was decided to investigate the incidence of hypothyroidism in the adolescent population in Ottawa by using as a screening test the determination of serum protein-bound iodine performed by two different methods.

A survey of the literature indicated that while normal values for protein-bound iodine in adults had been well-established,[1, 2] there were relatively few reports on adolescent groups and the numbers tested were small. A summary of the previously reported studies[3-8] on protein-bound iodine values in adolescent children is found in Table I.

We undertook to establish normal values for protein-bound iodine in adolescents by performing tests on a sufficient sample in the age range of 10 to 18 years inclusive and including an equal number of boys and girls. Correlations were to be sought between protein-bound iodine levels and various somatic changes associated with puberty and between protein-bound iodine levels and scholastic performance and/or intelligence.

In addition, the study was intended to serve as a pilot survey of the incidence of hypothyroidism in Ottawa school children in these age groups.

MATERIALS AND METHODS

Included in the study were 282 children (141 boys and 141 girls) from one secondary school and two elementary schools in Ottawa. The purposes of the study were explained to the students at a school assembly and volunteers were requested for testing. In each school between 45 and 50% of the students volunteered and obtained written parental consent. The volunteers were fairly evenly distributed throughout the various grades, and students included in the study were randomly selected from among the volunteers.

Each student was interviewed and briefly examined. The following information was recorded: Height and weight were measured by the school nurse, and each student was questioned as to his or her estimation of height and weight gain during the preceding year. Intelligence quotients were obtained where they had been measured; any with a numerical value were adjusted to conform with the A+, A, B, C+, C, C—, D and D— scale. Family history of thyroid disease or goitre was obtained by direct questioning, no attempt being made to distinguish between various forms of thyroid disease. Information concerning age at menarche, details of menstrual cycle and presence or absence of dysmenorrhea was obtained by direct questioning.

During the brief physical examination note was made of hair colour and body build as well as of voice changes in boys and the presence or absence of acne. As an indicator of the onset of secondary sexual characteristics the degree of hirsutism was noted by Dr. K. Rowan-Legg in the boys and by the school nurse in the girls, and was graded according to Tanner.[9]

All students were questioned about intake of medication, specifically regarding drugs known to affect protein-bound iodine values. Those in whom there was any doubt regarding interfering medication were excluded from the study group.

Each student then had 15 to 20 c.c. of venous blood withdrawn. The serum was separated within four hours after clotting and placed in frozen storage. All sera were tested within six months. Subsequent checking of several sera at intervals indicated that the duration of freezing within this limit had no significant effect on the protein-bound iodine values.

In most, but not all, of the 282 serum samples collected, the protein-bound iodine values were determined by two different methods. The Hycel chromic acid digestion (Hycel Corporation, Houston, Texas) was used in 235, and the Technicon AutoAnalyzer continuous perchloric acid digestion (Technicon International, Chauncey, New York) in 208.

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TABLE I.—PREVIOUS STUDIES OF SERUM PROTEIN-BOUND IODINE (PBI) AND BUTANOL-EXTRACTABLE IODINE (BEI) IN ADOLESCENT CHILDREN

Authors	*Year*	*PBI or BEI method*	*No. of children*	*Age ranges (year)*	*Results and comments PBI or BEI in μg./100 ml. ± s.d.*
Danowski, T. S. *et al.*[3]	1952	Barker (Mod) (PBI)	64 diabetic 64 normal	1-16 6-18	Mean 5.1 ± 1.0 Mean 4.7 ± 0.8 No significant differences between normal and diabetic children and between sexes. Significant lower PBI levels at 12-14 age range than in other age groups. There was a correlation between PBI levels and intelligence.
Politzer, W. M. and Munoz, J. A.[4]	1962	Meyer (PBI)	129 males 91 females	5-25	28 males and 16 females were below 4.0 μg./100 ml. These were Basuto children in an endemic goitre area. No real difference was noted between these children and goitrous children in the same area. No breakdown in sex and age difference was attempted.
Burrow, G. N., Hamilton, H. B. and Man, E. B.[5]	1962	Man (BEI)	46 males 35 females	14-15	Mean 5.2 ± 1.1 Mean 5.8 ± 1.1 There was a statistically significant difference between males and females but in neither sex was there a significant difference in BEI level when maturity was measured by epiphyseal closure.
Dreyer, D. R. and Man, E. B.[6]	1963	Man (BEI)	73 males	13-18	Range 2.9—5.9; no mean given. Boys in early adolescence showed values significantly lower than those of boys in late adolescence and in adults.
Fisher, D. A., Oddie, T. H. and Wait, J. C.[7]	1964	Barker (PBI)	prepubertal: 36 males 45 females postpubertal: 76 males 65 females	5-24	5.3 ± 0.8 5.3 ± 0.7 mean ± s.d. 4.8 ± 0.7 5.1 ± 1.0 Values were stated to be significantly lower in postpubertal males than in prepubertal males and in adults.
Rauh, J. L., Knox, M. D. and Goldsmith, R.[8]	1964	Barker (PBI)	96 children	10-18	Mean PBI: Males 4.9 Mean PBI: Females 5.3 It was stated that there was a significant drop in PBI values at the time of maximum somatic and sexual growth.

RESULTS

Ten children (3.6%) were found to have protein-bound iodine values outside the arbitrary normal range for one or other of the methods (3.5 to 7.5 μg. per ml. for Hycel and 4.0 to 8.0 μg. per ml. for Technicon AutoAnalyzer). These findings are summarized in Table II and in three instances indicate the considerable variation between the values obtained by the two methods on the same individual. These abnormal results were excluded from the remainder of the data analysis.

The protein-bound iodine values for males and females of various ages determined by each of the two methods are shown in Table III and Figs. 1 and 2. It is seen in all groups that results obtained by the AutoAnalyzer method were higher than for corresponding samples tested by the Hycel method. This is also apparent in the histogram of the frequencies of the results obtained by the two methods (Fig. 3). The overall correlation between the results obtained by the two different methods was good, the correlation coefficient (r) having a value of + 0.42 significance testing for r being $0.001 < P < 0.01$.

TABLE II.—ADOLESCENT CHILDREN WITH ABNORMAL PBI VALUES

Case	*Sex*	*Age*	*PBI in μg. per 100 ml. Hycel*	*AutoAnalyzer*
32	M	18	3.2	4.5
40	M	18	2.6	4.5
54	M	17	3.1	—
120	M	16	6.6	8.1
150	M	15	2.7	4.6
161	F	14	6.2	8.7
488	M	13	—	3.5
392	M	11	—	3.9
399	F	11	—	3.7
357	F	10	—	11.8

TABLE III.—AVERAGE PBI VALUES FOR MALES AND FEMALES BETWEEN THE AGES OF 10 AND 18 ESTIMATED BY TWO METHODS ON THE SAME SERUM

	Males				*Females*			
	Hycel		*AutoAnalyzer*		*Hycel*		*AutoAnalyzer*	
Age	*No. of children*	*Mean ± s.d. µg./100 ml.*	*No. of children*	*Mean ± s.d. µg./100 ml.*	*No. of children*	*Mean ± s.d. µg./100 ml.*	*No. of children*	*Mean ± s.d. µg./100 ml.*
18	19	4.7 ± .5	16	5.9 ± .6	21	4.9 ± .8	—	—
17	23	4.7 ± .9	21	5.8 ± .7	21	4.9 ± .7	—	—
16	20	4.7 ± .6	19	5.9 ± .6	22	5.4 ± .7	3	5.7 ± 1.1
15	21	4.6 ± .7	20	6.0 ± .6	23	5.3 ± .8	22	5.7 ± .6
14	21	4.5 ± .7	17	5.9 ± .7	21	5.1 ± .8	21	5.3 ± .9
13	9	4.6 ± .6	12	5.4 ± .8	8	4.9 ± .6	12	5.3 ± .8
12	—	—	5	5.5 ± .9	—	—	6	5.5 ± .6
11	—	—	6	5.3 ± .8	—	—	6	5.5 ± .7
10	—	—	7	5.1 ± .8	—	—	6	6.2 ± .6
Total	113	4.6 ± .7	123	5.8 ± .7	116	5.1 ± .7	76	5.5 ± .8

Table III shows that the protein-bound iodine values by either method did not differ significantly ($p > 0.05$) between any age groups or between boys and girls although there may be a tendency for the values to be lower in boys 13 and under than in boys 14 and older.

No significant correlation was demonstrable in either sex between weight and height gained in the year preceding the test and between the protein-bound iodine values obtained by either method (Table IV). The other indicators of development employed, such as beard, acne, body build and body hair, also did not show any correlation with protein-bound iodine values. In the girls, the values did not appear to be related to either the duration or the interval between menstrual periods and indeed were not different between premenarchal girls and those who had been menstruating for some time (Table V).

The intelligence quotient of those children in whom it was measured showed no relation to protein-bound iodine values obtained by either method (Table VI).

Attempts to correlate the most recent class examination marks with the protein-bound iodine similarly gave negative results.

In the 48 children (17%) who gave a family history of some form of thyroid disease, the mean protein-bound iodine values did not differ from the group as a whole (4.6 and 5.6 µg. per 100

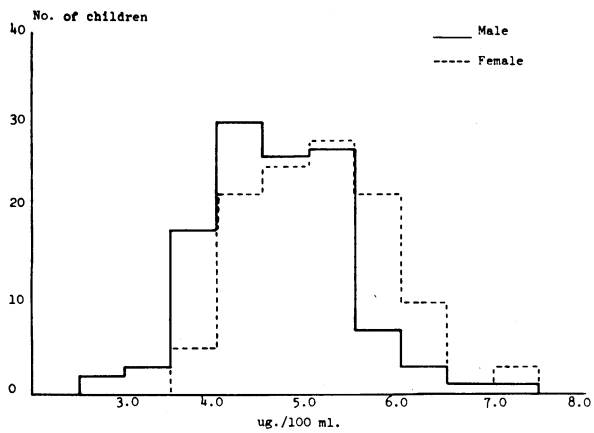

Fig. 1.—Distribution of protein-bound iodine values as estimated by the Hycel method on 234 children aged 10 to 18 years.

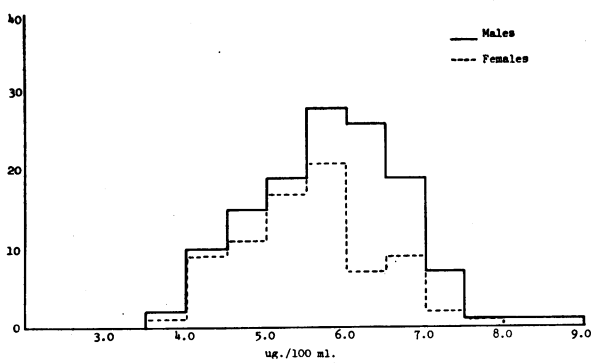

Fig. 2.—Distribution of protein-bound iodine values as estimated by the AutoAnalyzer method on 208 children aged 10 to 18 years.

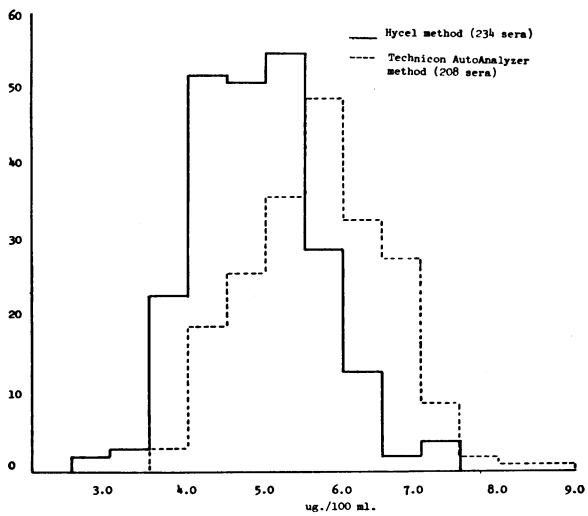

Fig. 3.—Comparison of protein-bound iodine distribution as performed by the two methods.

TABLE IV.—PBI LEVELS IN RELATION TO GROWTH RATE IN PRECEDING YEAR

Increase in height over year	Males				Females			
	Hycel		AutoAnalyzer		Hycel		AutoAnalyzer	
	No. of children	Mean µg./100 ml.	No. of children	Mean µg./100 ml.	No. of children	Mean µg./100 ml.	No. of children	Mean µg./100 ml.
0-¾"	54	4.6	54	6.1	84	5.1	29	5.4
1-1¾"	24	4.7	26	5.9	18	5.1	19	5.7
2-2¾"	21	5.7	25	5.6	8	5.0	16	5.5
3-3¾"	8	4.6	10	5.9	4	5.0	7	5.1
4-4¾"	3	4.5	4	5.5	1	5.1	3	5.7
5 +"	3	3.9	4	5.0	1	5.6	2	4.3

TABLE V.—RELATIONSHIP OF PBI TO MENSTRUAL PERIOD

	Hycel		AutoAnalyzer	
	No. of children	Mean µg./100 ml.	No. of children	Mean µg./100 ml.
Premenarchal	1	6.5	14	5.8
Postmenarchal	115	5.1	62	5.4
Menstrual cycle in days				
< 25	12	5.2	4	5.2
26 – 30	92	5.1	49	5.4
> 31	6	5.3	7	6.0
irregular	5	4.8	2	5.8
Menses in days				
< 3	—	—	—	—
3 – 6	90	5.1	49	5.4
> 6	25	5.0	13	5.5

ml. compared to 4.9 and 5.7 µg. per 100 ml. using the Hycel and AutoAnalyzer methods respectively).

TABLE VI.—PBI LEVELS AND INTELLIGENCE QUOTIENTS

I.Q.	Hycel		AutoAnalyzer	
	No. of children	Mean µg./100 ml.	No. of children	Mean µg./100 ml.
A+	15	4.5	16	5.8
A	26	5.0	34	5.8
B	36	4.9	41	5.4
C+	18	5.1	20	5.7
C	45	4.9	30	5.8
C−	16	4.9	15	5.8
D	24	4.9	15	5.8
D−	10	5.1	3	6.2

DISCUSSION

This study was undertaken, first to determine the incidence of abnormal thyroid function as measured by serum protein-bound iodine in a non-selected group of adolescents, and second to establish the normal range of protein-bound iodine values in the adolescent age range. Certain other factors were also considered which might possibly be associated with trends in protein-bound iodine levels.

The incidence of 3.6% abnormal protein-bound iodine values in non-selected school children was unexpectedly high. These results suggest that about one in every 30 school children in the 10 to 18 year age range in the Ottawa area might be expected to have a protein-bound iodine level consistent with disordered thyroid activity or with abnormal levels of thyroxine-binding proteins. The school children with abnormal values for protein-bound iodine were all euthyroid on clinical examination and as a group showed no consistent differences from the children as a whole in the various somatic, sexual and intellectual indices measured.

In Cases 32, 40, 54 and 150 in Table II, repeat blood samples were taken and PBI was measured by the Hycel method. In all cases the results were again abnormally low.

The factors known to affect protein-bound iodine levels have recently been reviewed in detail by Davis:[10]

Age: Shortly after birth, protein-bound iodine levels rise to "hyperthyroid" or high normal levels,[11, 12] then fall to the normal adult range within one or two months. In adults, there is some disagreement as to whether age affects protein-bound iodine values. Radcliff[2] noted a statistically significant lowering in subjects over 50 years of age, and Tucker[13] and Kountz, Chieffi and Kirk[14] noted lower values in older males than in younger males. Other authors, including Starr[15] and Gaffney *et al.*,[16] found no difference with advancing age.

Several authors[3, 7] have noted a decrease in protein-bound iodine levels at adolescence, which was more marked in males. However, Means, De Groot and Stanbury[12] stated that there was no change and Acland[17] reported a trend to increased levels.

Although a trend to lower protein-bound iodine values (Table III) in 10- to 13-year-old boys than in older boys was noted in our series, this difference was not statistically significant (as measured by the students' "t" test), nor was there a statistically significant difference between age groups in either sex.

Sex: No statistically significant differences in protein-bound iodine values attributable to the

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sex of the subjects, either in the group as a whole or in the various age groups, were found. It is apparent in Table III, however, that in most age groups there is a tendency for the mean values for females to be higher than for males. This finding was not unexpected in view of the well-known action of estrogen in raising the amount of thyroxine-binding globulin available. Burrow, Hamilton and Man[5] also found significantly lower levels in 15-year-old Japanese males than in 15-year-old females. In adults, Lowrey and Starr[18] found a significantly lower level of protein-bound iodine values in females than in males, but Means, DeGroot and Stanbury[12] noted no difference between the sexes. Margolese and Golub[19] noted that there was a cyclical variation in the day-to-day levels in both sexes, and that in females the levels tended to be higher and the oscillation in values greater in the luteal phase of the menstrual cycle.

Sexual and somatic development: The evidence concerning changes in values of protein-bound iodine with sexual and somatic development is conflicting. Danowski *et al.*[3] noted a trend towards lower protein-bound iodine values in early adolescence in both normal and diabetic children, but no attempt was made to relate these values to sexual or somatic growth, the term "early adolescence" being used by Danowski in the context of age alone. Dreyer and Man[6] noted a significant lowering of protein-bound iodine values during early stages of sexual development in males. Significantly lower values were noted by Rauh, Knox and Goldsmith[8] at the time of maximal somatic and sexual development in both sexes, although the differences were more significant in males. Fisher and his group[7] in Arkansas noted a marked decrease in levels in post-pubertal males. Burrow, Hamilton and Man[5] however, using epiphyseal closure as a measure of maturity in children of the same age group, noted no difference in butanol-extractable iodine values in either males or females when epiphyseal closure was used as the index of maturity. Malvaux *et al.*[20] also noted no difference in protein-bound iodine values between males at varying stages of sexual development or between adolescent and adult males.

Our results agree in general with those of the latter two authors. Taking the increase in height and weight over the past year as an index of somatic growth, no significant differences in mean protein-bound iodine values were found in either sex in children who had not started growing, in children who were growing rapidly and in children who had stopped growing. There is a decrease in thyroxine-binding pre-albumin with the onset of puberty,[6, 20] and one might expect this to be reflected in a lower protein-bound iodine level. Our failure to detect significant differences in this series could reflect lack of sensitivity of the methods used and/or insufficient sample size. However, it is planned to extend this series, using only the AutoAnalyzer method.

Intelligence: The effect of hypothyroidism in dulling intellect is well known, and it has been shown by Laroche, Milhaud and Vichnevsky[21] that goitrous children in endemic goitre areas perform less well scholastically than children in the same areas who do not have goitre. It was thus of interest when Danowski's survey[3] suggested that there might be a direct relation between intelligence and protein-bound iodine levels, those with higher intelligence quotients having higher protein-bound iodine values than those with lower intelligence quotients. However, in the larger group of children in the present series, this was not corroborated, because within the accepted normal ranges for protein-bound iodine values, no correlation is seen between intelligence and protein-bound iodine levels. These results are in agreement with those of Politzer and Hudson[22] and Simon *et al.*[23]

Thyroxine binding proteins: The major portion of the iodine measured in the protein-bound iodine estimation is derived from thyroxine bound to proteins in the blood plasma. The main thyroxine-binding proteins are thyroxine-binding globulin (TBG), thyroxine-binding pre-albumin (TBPA) and albumin, in decreasing order of affinity for thyroxine. It is obvious that any gross changes in the amount of binding proteins available or in their affinities for binding thyroxine will produce profound changes in the protein-bound iodine. These changes will not reflect the true thyroid status of the individual, since this is determined by the amount of free unbound thyroxine available at the cellular level and this is often normal.

Thyroxine-binding globulin is increased in amount during pregnancy and estrogen therapy, giving high protein-bound iodine values. Androgen therapy has the opposite effect. In nephrosis thyroxine-binding globulin is reduced, owing to renal leakage, and low protein-bound iodine values may be obtained. Certain drugs, including salicylates and diphenylhydantoin, displace thyroxine from the binding proteins, thereby also leading to low values. General illness and trauma can lead to a lowering of the protein-bound iodine due to a decrease in the available thyroxine-binding pre-albumin.

In the present study thyroxine-binding proteins were not measured, but at the original

interview all students were specifically questioned regarding drug intake and recent illness which might have affected the protein-bound iodine values.

Other Factors: No correlation was seen in this group between protein-bound iodine values and hair colouring, body build or menstrual history.

Summary The serum protein-bound iodine levels were determined in 282 children between the ages of 10 and 18 years using two methods of analysis—the Hycel method and the AutoAnalyzer method.

Ten children (3.6%) had protein-bound iodine values outside the normal range—3.5 to 7.5 μg. per 100 ml. for the Hycel method, or 4.0 to 8.0 μg. per 100 ml. for the AutoAnalyzer method. Seven of the 10 abnormal results were low.

In general, boys had slightly lower levels than girls, and boys under 14 had slightly lower levels than older boys, although these differences were not statistically significant. No statistically significant correlations were found between protein-bound iodine levels and growth rate, sexual development or intelligence.

Résumé Les auteurs ont calculé les valeurs de l'oide protéique chez un groupe de 282 enfants, dont l'âge variait de 10 à 18 ans. Ils ont, à cette fin, employé deux analyses différentes: la méthode Hycel et la méthode AutoAnalyzer.

Chez 10 enfants (3.6%), on a trouvé des valeurs d'iode protéique situées en dehors de la gamme normale, soit 3.5 à 7.5 μg./100 ml. d'après la méthode Hycel et de 4.0 à 8.0 μg./100 ml. d'après la méthode AutoAnalyzer. Sept des valeurs anormales étaient faibles.

En général, chez les garçons les valeurs étaient légèrement inférieures à celles des filles et, chez les enfants de moins de 14 ans, un peu plus faibles que chez les adolescents plus âgés. Ces differences ne tiraient pas à conséquence du point de vue statistique. Au même point de vue, on n'a trouvé aucune corrélation qui aît porté à conséquence entre les valeurs de l'iode protéique et le rythme de la croissance, le développement sexuel ou l'intelligence.

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