Serum Vitamin B_{12} Levels and Incidence of Tapeworm Anemia in a Population Heavily Infected with Diphyllobothrium Latum

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NEXT to Addisonian pernicious anemia, fish tapeworm anemia is probably the most thoroughly investigated of all the vitamin B_{12} deficiency states.[1,2] The anemia is characterized by macrocytosis and megaloblastic bone marrow, and is hematologically, neurologically and clinically identical with genuine pernicious anemia, from which condition it only differs in that the patients have intrinsic factor in their gastric juice. Moreover, spontaneous remission sets in when the worm is expelled.[1] That the condition is due to vitamin B_{12} deficiency has been proved beyond doubt by the demonstration that it is associated with low microbiologic serum vitamin B_{12} levels[3] and that parenteral administration of vitamin B_{12} causes a hematologic remission. The mechanism by which the deficiency state is brought about has recently been studied with radiovitamin B_{12} absorption tests.[4,5] In these, low absorption is observed as long as the worm remains *in situ*, whereas absorption is normalized after expulsion of the worm.

The incidence of tapeworm anemia in D. latum carriers has been believed to be relatively low. In 1926, it was estimated at 1:5,000 to 10,000 worm carriers,[6] but on the basis of more recent data, we have reckoned the figure to be tenfold, 1:500 to 1,000.[7] During the course of our radiovitamin B_{12} studies it became evident that in the majority of the *nonanemic* worm-carriers absorption was likewise inhibited, often being as poor as in patients with genuine pernicious anemia.[5] This observation made us suspect that vitamin B_{12} deficiency might be more frequent in tapeworm carriers than had hitherto been presumed. To study this possibility a research team was sent out to a heavily infected district in Eastern Finland to gather data regarding the incidence of anemia and to collect serum samples which later were assayed for their vitamin B_{12} content. Results obtained from this mass study are reported herein.

MATERIALS AND METHODS

For the field study the Lieksa district in Eastern Finland was chosen, because previous studies[8] had indicated that about 50 (42 to 61) per cent of its population was infected with D. latum. Some other reasons also dictated the choice: communications were relatively good, and owing to the efficiency of the local Red Cross branch, the population was favorably disposed toward this study.

Shortly before the field study began, a representative of the Red Cross visited all the schools in the area, delivering a speech to the pupils and distributing question forms. Every member of the pupils' families over nine years of age was to fill in the form and to visit temporary reception offices installed in schools and dispensaries bringing along a sample of feces. In addition, the inmates of the

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local old people's home were examined. A press campaign was also launched to bring in further population groups.

As a result of these efforts, about 80 per cent of those who received question forms presented themselves for examination. The total number of subjects examined was 1,345. The "school approach" explains why the youngest age group dominates the material. The research team consisted of two physicians (W.N. and M.S.), two laboratory technicians and a varying number of temporary personnel.

At the reception offices every subject was personally interviewed and a number of laboratory tests were performed. The stools were examined microscopically for parasite ova and a blood sample for vitamin B_{12} assay was taken into a thoroughly washed centrifuge tube. After clotting, the serum was separated by centrifugation. The serum specimens were frozen in boxes containing dry ice, transported further in the same boxes and finally stored in a deep-freeze at a temperature of $-25°$ to $-27°$c. Later their vitamin B_{12} concentrations were assayed with Euglena gracilis of the *z* strain, using the method of Hutner, Bach and Ross.[9] Some of the assays were performed as late as one year after collection of the samples. In our experience, the mean normal serum vitamin B_{12} level is within the range of 350 to 400 $\mu\mu$g./ml.

In addition to the examinations described, a number of hematologic tests were performed: determination of the erythrocyte count (visual counting), hemoglobin concentration (ordinary alkaline oxyhemoglobin method, readings with photoelectric colorimeter), volume of packed red cells (a disposable tube method[10]), and erythrocyte diameter (halometrically from smears). If clearly abnormal blood was found, the patient was persuaded to enter a hospital, or other arrangements were made to obtain a complete hematologic examination. This included bone marrow biopsy and radiovitamin B_{12} absorption tests.

Every subject was assigned a number, and the persons performing the various laboratory examinations never saw the name of the patient and did not know of the results of the other examinations. The material was not brought together until all the studies were completed, when all the data were transferred to punch cards. Exceptions to this rule were the clearly anemic patients who were examined more carefully during the field study.

In addition to the data referred to, the patients filled in the question forms which dealt with food habits and possible symptoms of worm infection.

TABLE I
Incidence of a Positive Finding of Tapeworm Ova in the Feces

Age Group (yr.)	Number of Subjects Examined			Per cent with Tapeworm Ova		
	Males	Females	Both Sexes	Males	Females	Both Sexes
9–15	216	308	524	14.8	19.8	17.7
16–30	63	111	174	27.0	26.1	26.4
31–40	44	122	166	29.5	29.5	29.5
41–50	83	136	219	44.6	29.4	35.2
51–60	61	77	138	41.0	31.2	35.5
61–70	24	25	49	41.7	32.0	37.5
71–89	27	48	75	55.6	39.6	45.5
All cases	518	827	1,345	28.8	26.2	27.2

This part of the material has not yet been examined in detail and will be published later.

Most of the data reported herein, as well as their statistical assessment, were obtained directly from the punch cards by means of computers (IBM Calculator 602-A and Accounting Machine 421).

RESULTS

Incidence of Tapeworm Ova

The incidence of tapeworm ova in the feces is reported in Table I. It is seen that the incidence increases with age, being highest in the oldest age group. The over-all frequency is 27.2 per cent, and no clear-cut difference is seen between the sexes.

Since the finding of tapeworm ova in the feces is a sure sign of the presence of the worm, failure to find ova in one sample of feces does not rule out the possibility of actual or recent worm infection, these figures represent minimum values. Furthermore, although comparisons are made between the subjects in whose feces tapeworm eggs were found and those in whom ova were not detected, this must not be interpreted as implying that the latter represent *healthy* control subjects.

Incidence of Anemia

A hemoglobin concentration lower than 11 gm./100 ml. was taken as the criterion of anemia. Such values were found in twenty-

TABLE II

Hematologic Data Relating to the Patients with Verified or Suspect Vitamin B_{12} Deficiency Anemia

Age (yr.)	Hemoglobin (gm./100 ml.)	Red Blood Cells (millions)	Hematocrit (per cent)	Mean Corpuscular Hemoglobin	Mean Corpuscular Volume (cu.μ)	Serum Vitamin B_{12} ($\mu\mu$g./ml.)	Bone Marrow*	Schilling Test† (per cent of oral dose)	
								Before Worm Expulsion	After Worm Expulsion
A. Subjects with Tapeworm Ova in the Feces									
25	6.0	1.56	20	38	128	57	+	0	10.5
23	10.5	3.28	37	32	113	30	+	2.1	27.3
45	6.5	1.89	21	34	111	16	+	0	8.4
56	6.6	1.52	19	43	125	28	+	0	15.1
60	9.9	2.44	31	41	127	20	+	0	9.2
51	10.9	2.85	36	38	126	1	+	1.1	–
69	6.6	1.85	21	36	114	20	+	0	11.9
50	8.8	3.36	33	26	98	77	+?	–	–
25	9.5	3.82	36	25	94	71	+	–	–
42	10.2	3.72	37	27	99	53	–	–	–
14	10.5	4.38	–	24	–	53	+?	–	–
38‡	8.5	2.72	26	31	96	273	+	–	–
B. Subjects in Whose Feces Tapeworm Ova Were Not Found									
40	9.9	3.48	37	28	106	40	+?	–	–
42	10.2	3.20	33	32	103	54	+?	–	–
79	10.5	4.23	35	25	83	62	+?	–	–

* Code: + megaloblastic, +? macroblastic without typical megaloblasts, – normal.

† Standard procedure with 1 μg. oral dose, as described by Gräsbeck.[13]

‡ This patient was currently being treated with vitamin B_{12} and a liver preparation given orally and had received a vitamin B_{12} injection one week before this examination.

two worm carriers (6 per cent) and in twenty-eight controls subjects (2.9 per cent). In the worm-carrier group seven anemic subjects (1.9 per cent of the carriers) can be regarded as having indubitable vitamin B_{12} deficiency anemia with typical blood and bone marrow changes, low serum vitamin B_{12} level and Schilling test values lower than 2.5 per cent (Table II). In addition, there was one other patient with megaloblastic anemia who had quite recently been treated with vitamin B_{12}, which probably explains why the serum vitamin B_{12} concentration was normal. When the results of the vitamin B_{12} assays were ready, it was found that another four anemic worm carriers (1.1 per cent) had low serum vitamin B_{12} levels. Since the anemia was hypochromic, vitamin B_{12} deficiency was not suspected during the field study and Schilling tests were not performed. If these suspect cases are added to the verified cases of vitamin B_{12} deficiency anemia, altogether 3 per cent of the worm carriers had a combination of low vitamin B_{12} level and anemia.

Among the control subjects, only three anemic subjects had vitamin B_{12} levels lower than 100 $\mu\mu$g./ml., representing 0.3 per cent of the control group. The anemia in these cases was macrocytic, but the presence of megaloblasts in the bone marrow could not be demonstrated with certainty.

It is worth mentioning that in none of the accidentally discovered eight cases of megaloblastic anemia was the subject aware of being ill. If these eight cases are taken as a minimum value for the incidence of tapeworm anemia, a frequency of about one case in fifty worm carriers is obtained.

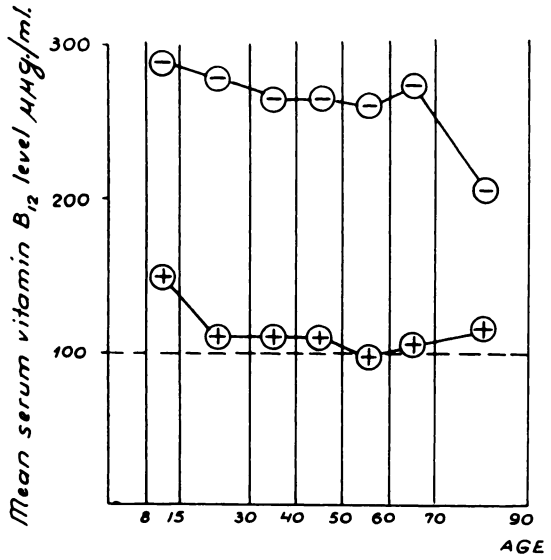

FIG. 1. The mean serum vitamin B_{12} concentrations in the different age groups of the tapeworm carriers (+) and those in whose feces tapeworm ova were not observed (−).

Microbiologic Serum Vitamin B_{12} Concentration

The mean vitamin B_{12} levels in the worm carrier and control groups were 116.4 and 273.8 µµg./ml., respectively. The mean values for the age groups are seen in Figure 1.

Since the presence of anemia may have prejudiced the investigators (these patients received more detailed examination than the rest of the subjects) and the anemia in itself may have had an effect on the serum vitamin B_{12} concentration, the following analysis was made on a reduced series, from which the anemic cases had been omitted.

For the statistical treatment the material was divided into "blocks," consisting of subjects of the same age and sex, and belonging to one of two hemoglobin concentration groups (hemoglobin 11 to 13.9 and ≧14 gm./100 ml.). When the comparisons are made within these blocks, the possibly different distributions of age, sex and hemoglobin concentration in the groups with or without tapeworm ova should have no influence on the result. The serum vitamin B_{12} concentrations in these blocks are given in Table III.

It is seen that without exception the mean vitamin B_{12} concentration was lower in the tapeworm-carrier group. This finding in itself is sufficient to demonstrate the existence of a statistically significant difference.[11] However, the high level of significance becomes more evident when a normal comparison of the means is made within the blocks and the evidence obtained from these twenty-eight comparisons is combined into an over-all significance. This was performed by analysis of variance. For this purpose two estimates for the natural variance were computed, one for the variation between the means in the groups with or without tapeworm ova with 28 degrees of freedom, the other estimate for the variation within the fifty-six blocks with 1,217 degrees of freedom.

The variance ratio $F(28,1217) = 192,548/19,759 = 9.74$ exceeds unity so greatly that the significance level is over 99.95 per cent (for which a variance ratio of 2.10 would have sufficed).

If the differences between the mean microbiologic vitamin B_{12} concentrations of the worm carriers and the control subjects belonging to the same blocks are compared (Table III), it is noted that they are of the same order of magnitude. The median of these differences is 150 µµg./ml. If the assumption is made that the presence of the worm decreases the serum vitamin B_{12} level by 150 µµg./ml., and this figure is added to all the observed concentrations of the worm carriers, the entire material becomes homogenous. An ordinary comparison between the mean concentrations in the fifty-six blocks resulting (variables: sex, age, hemoglobin, presence of tapeworm ova; $2 \times 7 \times 2 \times 2 = 56$ blocks) shows that the differences between the blocks can be explained on the basis of natural variance [$F(55,1217) = 1.20$].

Effect of Age, Sex and Hemoglobin on the Serum Vitamin B_{12} Level

A similar statistical analysis was performed to elucidate the influence of the variables age, sex and hemoglobin (≧11 gm./100 ml.) on the serum vitamin B_{12} concentration. In dividing into blocks the variable presence or absence of tapeworm ova was taken into account. The variance ratios do not indicate statistically significant effects of age, sex or

TABLE III

Mean Serum Vitamin B_{12} Levels in "Blocks" Consisting of Subjects of the Same Age and Sex and One of Two Hemoglobin Concentration Groups

Block			Control Subjects		Worm Carriers		Difference Between Mean Vitamin B_{12} Levels of Control Subjects and Worm Carriers ($\mu\mu$g./ml.)
Age Group (yr.)	Sex	Hemoglobin*	No.	Mean Serum Vitamin B_{12} Level ($\mu\mu$g./ml.)	No.	Mean Serum Vitamin B_{12} Level ($\mu\mu$g./ml.)	
9–15	F	L	168	284.9	43	141.3	143.6
		N	76	293.7	15	157.4	136.3
	M	L	120	269.3	21	166.9	102.4
		N	60	274.3	11	116.0	158.3
16–30	F	L	50	290.4	18	114.7	175.7
		N	30	261.6	9	72.2	189.4
	M	L	7	187.9	6	94.2	93.7
		N	39	289.2	9	162.1	127.1
31–40	F	L	55	256.7	23	95.9	160.8
		N	23	337.4	8	91.0	246.4
	M	L	1	185.0	6	95.7	89.3
		N	30	236.9	7	100.7	136.2
41–50	F	L	59	271.4	19	108.2	163.2
		N	27	288.4	16	93.8	204.6
	M	L	11	255.2	10	133.6	121.6
		N	35	226.2	27	105.1	121.1
51–60	F	L	26	255.4	12	99.3	156.1
		N	25	271.2	8	100.6	170.6
	M	L	11	215.8	9	88.1	127.7
		N	24	276.8	15	110.4	166.4
61–60	F	L	10	239.2	4	119.8	119.4
		N	7	253.1	3	73.3	179.8
	M	L	3	240.3	1	23.0	217.3
		N	11	319.2	9	122.7	196.5
71–89	F	L	4	274.8	7	107.6	167.2
		N	17	195.6	7	128.0	67.6
	M	L	4	191.0	6	67.3	123.7
		N	5	226.0	6	88.7	137.3
			938†		335†		Median = 150

* Code: N(ormal) = hemoglobin ≧ 14; L(ow) = 11 — 13.9 gm./100 ml.

† This is a reduced series; patients with hemoglobin below 11 gm./100 ml. are not included. In addition, a few patients were eliminated because personal or laboratory data were lacking.

hemoglobin in the normal range on the serum vitamin B_{12} concentration.

Incidence of Low Serum Vitamin B_{12} Levels

The effect of D. latum on the serum vitamin B_{12} level is perhaps best illustrated by the incidence of low serum vitamin B_{12} concentrations in the worm carriers. It is generally recognized that serum vitamin B_{12} concentrations below 100 $\mu\mu$g./ml. are pathologic, at least when the assay is performed according to our present technic.[2,12,13] The incidence

TABLE IV

Number of Patients with Serum Vitamin B_{12} Concentrations below 100 $\mu\mu$g./ml. Among Nonanemic (Hemoglobin $\geqq$ 11 gm./100 ml.) Tapeworm Carriers and Control Subjects

Age Group (yr.)	D. latum Carriers		Subjects with No Tapeworm Ova	
	No.	Per cent	No.	Per cent
9–15	93	32.6	431	4.4
16–30	46	53.5	128	3.2
31–40	49	63.6	117	5.5
41–50	77	56.9	142	3.0
51–60	49	56.8	89	6.9
61–70	18	47.1	31	6.7
71–89	34	70.0	41	16.7
Whole material	366	51.6	979	4.9

of values below this level in the nonanemic subjects is reported in Table IV. It is seen that low values were observed in 51.6 per cent (!) of the worm carriers, but only in 4.9 per cent of the control subjects. If the different age groups are compared, low values were significantly (at 99.9 per cent level) less frequent in the youngest age group than in the oldest age group, whereas the other age groups did not differ significantly from each other. Among the control subjects no significant differences between the age groups could be observed.

COMMENTS

The population studied was selected because it was expected that about 50 per cent harbored D. latum. The incidence was found to be only 27.2 per cent, however. Because healthy people would be less likely to respond to our appeal than worm carriers, the true incidence may be even lower. The apparent drop in the incidence (chiefly involving the young age groups) which seems to have occurred since the second world war may be misleading and due to the fact that the earlier estimate[8] was based on indirect observations, whereas the present investigation is the first one in which a true field study has been performed.

However, the best explanation of the change in the incidence is probably the marked improvemenet in hygienic standards which has taken place in the region since the war. This is partly the result of anti-worm propaganda on the part of the local authorities, but perhaps even more due to the general rise in living standard and the industrialization of the region which have changed the dietary habits of the population, especially those of the children and adolescents. For instance, much of the fish eaten is nowadays bought salt-water fish, whereas earlier most had been caught in near-by lakes by the families themselves.

The data demonstrate very clearly that the presence of D. latum markedly decreases the serum vitamin B_{12} level in all age groups. The incidence of tapeworm anemia appears to be at least one case in fifty worm carriers, a figure ten to twenty times higher than has hitherto been suggested.[7] It is quite astonishing that more than half the worm carriers had a pathologically low serum vitamin B_{12} level.

To what extent the serum vitamin B_{12} level reflects the amount of vitamin B_{12} stored in the body is uncertain, although many facts indicate that there is a good correlation between the two, at least when dysproteinemia, liver damage or folic acid deficiency do not complicate the picture.[13] However, it seems certain that a high percentage of the worm carriers suffer from relative, subclinical vitamin B_{12} deficiency. The effects of this subclinical deficiency on health cannot be assessed at the present moment, but harmful effects on fetal development, child growth and general resistance toward infection may be imagined. It is hoped that further analysis of the collected material will shed light on these questions. Since more than 100,000 worm cures are taken per year and several hundred thousand D. latum carriers have been estimated to exist in Finland,[7] which has a population of about 4.4 million, large population groups would appear to suffer from subclinical vitamin B_{12} deficiency. It may therefore be conjectured that D. latum infection will prove to be a much greater public health problem than has hitherto been assumed.

SUMMARY

In order to elucidate the incidence of D.

latum infection, tapeworm anemia and low serum vitamin B_{12} levels, a population in Eastern Finland was subjected to a mass examination. Of the 1,345 subjects examined, 27.2 per cent were found to have tapeworm ova in their feces. Among those who had no tapeworm ova 0.3 per cent had a combination of anemia and low serum vitamin B_{12} level, but none had megaloblastic bone marrow. Among the worm carriers 1.9 per cent had verified (megaloblastic), and another 1.1 per cent suspect (nonmegaloblastic) vitamin B_{12} deficiency anemia. The incidence of tapeworm anemia in worm carriers is at least 1:50. The mean serum vitamin B_{12} levels in the tapeworm carrier and control groups were 116.4 and 273.8 $\mu\mu$g./ml., respectively, the difference being statistically highly significant. More than fifty per cent of the worm carriers had a serum vitamin B_{12} concentration below 100 $\mu\mu$g./ml., whereas this occurred in less than 5 per cent of the control subjects. It is concluded that the incidence of tapeworm anemia is much higher than has hitherto been supposed, that vitamin B_{12} deficiency is extremely common in D. latum carriers and that the D. latum endemy in Finland is thus to be regarded as a serious public health problem.

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