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SERUM LACTATE DEHYDROGENASE PATTERNS AND ARGINOSUCCINIC ACIDURIA IN ORDINARY ALOPECIA AREATA AND ALOPECIA DIFFUSA

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Walker and Rothman (13) in 1950 pointed to the lack of systematic investigations of the problem of hair loss. Since then several publications have appeared on both clinical and experimental studies. So far there is no apparent understanding of the pathogenicity of the different forms of hair loss, except in the case of aminogenic alopecia, a type of hair loss described by Shelley and Rawnsley (10). In this disease the protein synthesis in hair follicles is compromised due to incomplete conversion of arginosuccinic acid (ASA) which occurs as an intermediate metabolite in the Krebs-Henseleit urea cycle. Normally there is a continuous conversion into arginine and fumaric acid. If, however, there is a lack of the relevant cleavage enzyme, there is an accumulation of ASA in blood and tissues, and the substance can be found in the urine.

Other authors have described (1, 6, 7, 11, 15) the finding of ASA in the urine of patients with trichorrhexis nodosa and monilethrix, both as isolated symptom complexes and in connection with mental deficiency.

Concurrently with another investigation (4) of lactate dehydrogenase isoenzymes (iso-LDH), we have examined the iso-LDH pattern in the blood from patients with alopecia areata and alopecia diffusa (defluvium cappillorum diffusa). As described by Vesell (12) markedly changed iso-LDH patterns can be found in various malignant, organo-specific or systemic diseases, even though the total LDH in serum is within its normal limits.

In the medical literature there are numerous descriptions of hair loss attributed to general infections, malignant diseases, endochrine and nutritional disorders, intake of chemicals or antimitotic agents (2, 3, 8, 9). The authors of this article considered that one might therefore find an earlier unnoticed connection between hair loss and some of the other mentioned forms of disease by iso-LDH analyses of serum.

The urine from a number of patients has been examined for ASA, in order to get an impression of whether the abnormal protein synthesis, mentioned above, is a common phenomenon in patients with hair loss. Ordinary blood tests and urinalyses were also made on all patients whose case history or clinical appearance aroused suspicion of complicating disease. Seroreactions for syphilis were carried out on all the patients.

Materials and Methods

The material consists of data from 36 patients with alopecia areata, 40 with alopecia

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Table 1. *Age and sex distribution*

Age in years	Alopecia areata		Alopecia diffusa	
	Women	Men	Women	Men
0– 9	–	1	–	–
10–19	5	2	1	3
20–29	4	–	–	2
30–39	4	1	2	–
40–49	7	1	7	–
50–59	5	1	9	–
60–69	3	–	9	–
70+	2	–	7	–
Total	36		40	

Table 2. *Duration from onset of symptoms until present examination*

	Alopecia areata	Alopecia diffusa
0– 6 months	11	21
6–12 months	5	5
1– 2 years	–	1
2– 3 years	4	1
3– 5 years	2	1
5–10 years	–	2
10+ years	12	6
Do not know	2	3
Total	36	40

diffusa and 30 healthy controls. Age- and sex-distribution is shown in Table 1. No statistical screening of the patients or controls was done. For practical purposes only patients were examined who were seen during the course of daily routine work. The majority of the patients examined were women, since more women than men seek treatment for hair loss.

In the two groups of patients with hair loss there were both cases with recently developed and long standing symptoms. As shown in Table 2, the duration of illness for the majority (26/40) of patients with alopecia diffusa was less than one year, and for most of them there was a noticeable improvement of their condition in the period of observation. Consequently it does not seem likely that the hair loss seen was due mainly to age, in spite of the fact that there was a relatively large number of elderly women in this group.

No attempt has been made to group the patients according to analysis of "plucked hairs" and an estimation of the Anagen/Telogen ratio. However, it has appeared from the case histories that none of the patients suffered recently a serious infection or other known complicating disease. Furthermore, none of the patients had undergone treatment with any medicine known to cause hair loss. Finally, post partum alopecia was excluded.

The venipuncture blood samples were left standing at room temperature for 1/2–1 hour for coagulation, after which centrifugation was carried out at 3200 rpm (about 1500 G) to separate the serum. This was then pipetted off and kept at +4°C for 18–20 hours, until iso-LDH analysis could be carried out. Some samples were kept at room temperature, but control analyses made on samples from 10 patients in each of the two groups showed that storage at room temperature was without significance for the results. Control analyses also were carried out on samples from a similar number of patients immediately after centrifugation as well as after the usual 18–20 hours delay. This, also, was shown to be without importance for the results of the analyses.

The iso-LDH analyses were carried out by agar gel electrophoresis and subsequent incubation of the electrophoresed slides with an indicator medium at 37°C to demonstrate the activity and localization of the iso-enzymes according to Wieme (14) as described in our earlier publication (4). The only difference is that 10 μl of serum was used instead of supernatant from homogenised tissues. The iso-LDH enzymes encountered have been numbered LDH_{1-5}, where LDH_1 denotes the fastest migrating, most anodic and LDH_5 the slowest migrating, most cathodic iso-enzyme.

Total LDH analyses have been carried out according to the method described by Wroblewski and LaDue (16). This method measures the enzyme activity spectrophotometrically by recording the fall of NADH

Table 3. *Total serum-LDH and serum iso-LDH analyses in 30 healthy controls, 36 patients with alopecia areata and 40 patients with alopecia diffusa*

	Total LDH arbitrary units	LDH_1 relative %	LDH_2 relative %	LDH_3 relative %	LDH_4 relative %	LDH_5 relative %
Healthy controls						
Mean	6.5	50.2 ± 6.2*	28.0 ± 5.3	15.1 ± 3.0	3.6 ± 1.3	4.0 ± 1.8
Range	3–12	39–64	20–40	9–20	1–6	1–9
Alopecia areata						
Mean	7.0	53	29	14	3	2
Range	4–9	31–68	19–41	3–25	0–5	0–5
Alopecia diffusa						
Mean	7.0	51	30	15	3	3
Range	5–12	35–70	22–37	7–22	0–7	0–7

* Standard deviation.

(reduced form of Nicotineamide Adenine Dinucleotide) at 340 mμ. Normal values are 3–12 units.

The ASA analyses were carried out by a commercial laboratory according to the principle described by Dent (5). By this method urine was examined by two-way paperchromatography after desalting. The analyses were made 18–20 hours after the urine samples had been collected. Control analyses showed that with the method of analysis employed this delay is without importance for the evaluation of this semi-quantitative analysis.

Since Grosfeld and Mighorst (6) reports that they could not always demonstrate ASA in the urine from their patients when the samples were collected from fasting patients, early in the morning, we have taken all samples from non-fasting patients, between 10 a.m. and 1 p.m.

Results and Discussion

A. *Iso-LDH analyses*

As can be seen from Table 3 there is no significant difference in the mean values of the iso-LDH analyses of serum from the 30 healthy controls and the two groups of patients examined. Furthermore, most of the individual analyses of patients fall within the limits of the mean of the normal controls ± twice the standard deviation. In all instances where analyses from patients with hair loss fall outside these limits, the LDH_1 value is abnormal, so for clarity's sake the LDH_{2-5} values are disregarded in the following discussion.

Six patients with alopecia areata and two with alopecia diffusa were found to have abnormal LDH_1 values. Of these one patient with alopecia areata had a low LDH_1 value on repeated occasions, whereas the remaining patients all showed increased values. No connection was found, however, between the results of the iso-LDH analyses and the type, degree or duration of the hair loss. Neither was it possible to show any relationship to complicating diseases or to pathological laboratory tests, e.g. increased sedimentation rate.

B. *Total LDH analyses*

None of the patients examined showed abnormal total serum-LDH. This is also true for the patients who showed abnormal iso-LDH patterns. In our opinion this indicates that there is no relationship between serum-LDH and the forms of hair loss examined. It stands to reason that if a relationship existed, an increase in serum-LDH or a pathological iso-LDH pattern should be found either in patients with newly developed or long-standing symptoms.

C. *Arginosuccinic acid Urinalyses*

Two-way paperchromatography was performed on 22 patients with alopecia areata and 15 patients with alopecia diffusa. These tests showed no increase in the amino acids normally found in the urine and no trace of ASA except in one case of acute alopecia areata in a 26-year-old male. In this case approximately 250 mg ASA per liter urine was found, but this finding could not be confirmed on subsequent examinations. In this patient's family history there were no known cases of mental deficiency or premature hair loss.

Only the first half of the patients with hair loss was examined for the occurrence of ASA in the urine. Further testing was discontinued since the results were considered entirely negative.

D. *Other Investigations*

All patients were examined by serological tests for syphilis. All tests were found to be negative.

Hemoglobin analyses were made on three-fourths and granulocyte- and differential-counts on about half of the patients. Neither of these tests showed any important abnormalities. Furthermore, all protein-bound iodine, tri-iodinethyronine and serum cholesterol tests taken were within normal limits. Urinalyses were also all normal, except in two cases where moderate proteinuria due to urinary infection was found.

Sedimentation rates were determined in 25/36 of the patients with alopecia areata and 30/40 of those with alopecia diffusa. In the first group only four were found to have increased values. In the latter group, however, seven patients were found to have an increased sedimentation rate. This is perhaps not unexpected in a group which includes several elderly people.

SUMMARY

Iso-LDH and total LDH analyses of serum were carried out in 36 patients with alopecia areata, 40 patients with alopecia diffusa and 30 healthy controls. Paperchromatographic examinations of the urine for arginosuccinic acid were also made in approximately half of these patients. The results suggest these examinations to be without value in the routine testing of patients with one of the ordinary types of hair loss.

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