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Research Article

CYSTINE CONTENT OF FINGER NAILS IN PELLAGRA[1]

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An abnormality in sulfur metabolism in pellagra was first demonstrated by Camurri (1) and Myers and Fine (2). Indicanuria (3, 4), a decreased excretion of thiocyanate (5), and an increase in the excretion of absolute amounts of ethereal sulphates in the urine (2) of pellagrins have been observed. A reduction of sulfur in brain and spinal cord of five individuals succumbing with pellagra was reported by Koch and Voegtlin (6). The total amount and per cent cystine of rat hair decreases when these animals are fed diets deficient in vitamin G (7, 8).

It has been suggested (9) that the relatively high concentration of cystine in wool and hair is of physiologic importance in the protection of the organism against harmful effects of prolonged exposure to sunlight. The protecting effect of cystine solutions to paramecia exposed to ultraviolet rays was demonstrated by Harris and Hoyt (10) and experiments of Ward (11) seem to indicate that although several amino acids give marked general absorption bands, cystine is the only amino acid that has any marked absorption in the ultraviolet region of solar light. The high concentration of cystine in epidermal tissues of higher forms of animal life (12, 13), a content greater than that of any other tissue, suggests a possible specific protective function in this tissue. In the hope that we might find more experimental evidence for the clinical observation (14, 15) that skin lesions in pellagra indicate a light-sensitive condition, 40 cases of pellagra and 61 subjects without evidence of pellagra have been studied. Because of the technical difficulties in obtaining representative samples of skin we concluded that finger nails gave more uniform data from which to judge cystine content in epithelial tissue.

METHODS

In reviewing the available data concerning the sulfur and cystine content of the common keratins, one is impressed with the wide variation of the results in the hands of different observers. Much of the data is open to criticism in that no mention is made concerning the method of preliminary treatment, and variations in this may account for many of the discrepancies. Our preliminary work showed that if nails are thoroughly cleaned and the technique of drying standardized, there is little variation in cystine in nails from various fingers of the same individual. However, in spite of an attempt to subject each sample of

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nail parings to standard conditions of cleaning and drying, it was realized that some variation in the rate of loss of water from different samples was unavoidable. Therefore, the total nitrogen content (representing protein) and the N : S ratio were determined in a further effort to minimize the errors inherent in a series of determinations based on the dry weight of biological material.

Finger nail parings were soaked in water for 24 hours, cuticle and dirt removed by scraping, and after washing in running water dried in an oven for 24 hours at 90° C. Cystine was determined by a modification of the Sullivan method (16). From 30 to 60 mgm. clean dry finger nail parings and 5 cc. of 20 per cent hydrochloric acid were placed in a 50 cc. acetylation flask with a ground glass fitted reflux condenser. The flask was placed in a cold mineral oil bath and the bath brought to 120° C. at which heating was continued for 12 hours. The hydrolysate was transferred to a 25 cc. volumetric flask and the acetylation flask rinsed with 5 cc. of 0.1 N hydrochloric acid. The hydrolysate was treated drop-wise with 20 per cent sodium hydroxide until greenish to bromphenol blue, i.e., about pH 3.5. Dilution was made to 25 cc. with addition of 0.1 N hydrochloric acid. Colorimetric estimation of cystine proceeded according to Sullivan's method. Color matching is facilitated by the use of a yellow light filter glass disc devised for the Folin and Malmros (17) micro method for blood sugar determination.[2]

Total nitrogen of finger nails was determined by the Koch-McMeekin method (18) on the above hydrolysate.

Because of the slow and variable growth of the finger nail only cases with chronic disease have been considered and only stable conditions of at least two months duration have been included in the data. Thirty-three of the pellagrins were negro patients at the State Hospital at Goldsboro, North Carolina,[3] and were carefully followed for months. Several had experienced "recurrent" pellagra for years and had been observed at the institution over long periods of time. All gave histories of faulty dietaries for months, and three mental cases were observed to develop symptoms during self-inflicted periods of starvation. Treatment in all cases was chiefly dietary and followed the suggestions of Goldberger et al. (19, 20). High vitamin foods, especially milk and fresh meats, fruits, and vegetables, were given in abundance. Since many of the pellagrins were known to have syphilis on the basis of history of infection or positive blood Wassermann tests, data are presented from a control group of 20 cases with latent syphilis. Also since emaciation is common among pellagrins five malnourished cases of pulmonary tuberculosis are included.

Cystine content of normal finger nails

Only a few values for the cystine or sulfur content of normal human finger nails are available. The early work of Mulder (21), in 1847, showed human nail to contain 2.8 per cent sulfur corresponding to 10.5 per cent cystine. Buchtala (13), in 1907, pooled the finger nails from 16 cadavers and by a method which determined sulfur by sublimation, estimated cystine content to be 5.15 per cent. Langecker (22), in 1921, reported values for sulfur in finger nails from six patients with various diseases. His values range from 2.59 to 3.12 per cent corresponding to estimated cystine values

[2] Manufactured by the Klett Manufacturing Company, New York City.

[3] The authors are indebted to the Superintendent, W. Linville, M.D., and his staff for the opportunity to study and to obtain samples of finger nails from these patients.

of 9.7 to 11.7 per cent. Sullivan and Hess (23), in 1932, reported cystine content of the finger nails of normal individuals to vary from 11 to 13 per cent. Since the early methods involved the isolation of cystine from a hydrolysate and determination of precipitated sulfate, one would expect higher values in a colorimetric method such as Sullivan's.

In our analyses of finger nails from 36 healthy young adults, chiefly medical students and laboratory workers, Figure 1, *A* and Table I, cystine

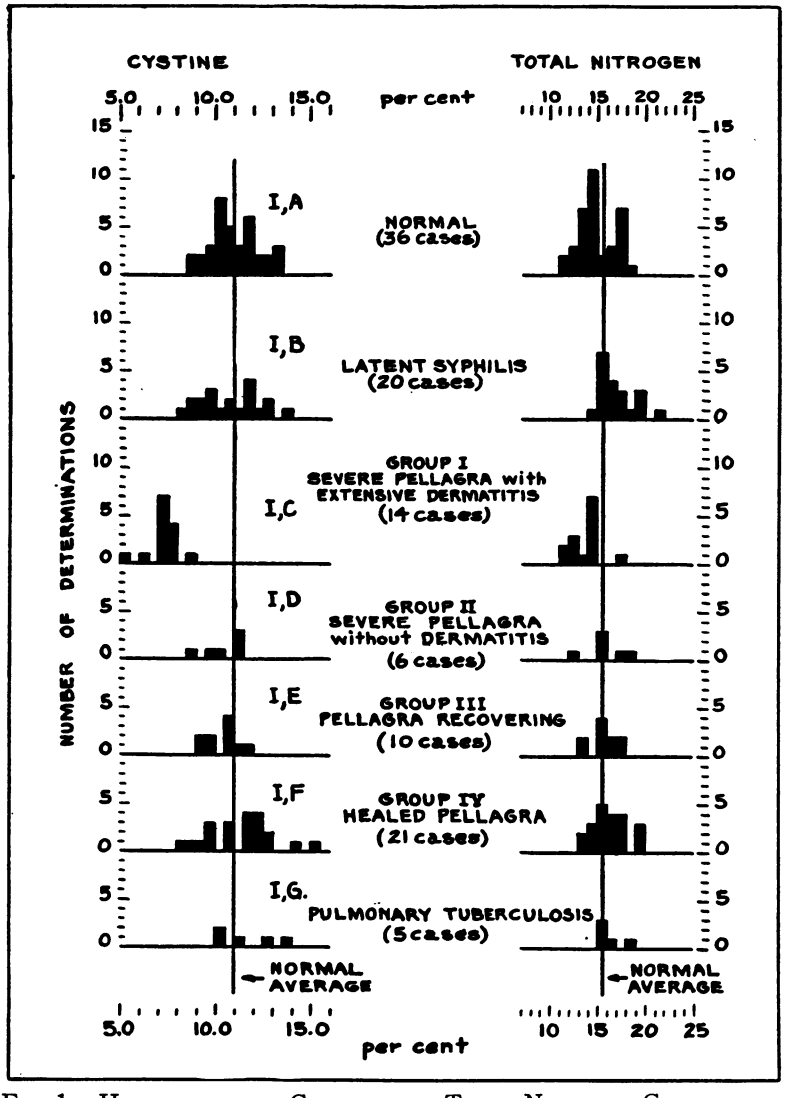

FIG. 1. HISTOGRAMS FOR CYSTINE AND TOTAL NITROGEN CONTENT OF THE FINGER NAILS IN NORMAL SUBJECTS AND IN PATIENTS WITH PELLAGRA, SYPHILIS, AND TUBERCULOSIS.

Each square represents a single determination.

TABLE I

Summary of cystine, total nitrogen, and N : S ratio in finger nails

	Number of cases	Cystine			Total nitrogen			N : S ratio *		
		Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Normal	36	8.77	13.38	10.97	12.6	18.7	15.8	4.61	5.92	5.40
Pellagra										
Group I										
Severe with dermatitis	14	5.03	8.55	7.26	12.6	18.1	14.8	6.27	11.27	7.63
Group II										
Severe without dermatitis	6	8.93	11.98	10.61	12.6	18.3	16.2	4.71	6.17	5.71
Group III										
Healing	10	9.26	11.93	10.47	13.5	17.6	15.5	4.57	6.16	5.56
Group IV										
Cured	21	8.22	15.42	11.32	13.6	19.8	16.3	4.53	7.43	5.40
Syphilis	20	8.20	13.55	10.64	14.1	21.9	16.9	4.81	7.11	5.95
Tuberculosis	5	10.28	13.64	11.66	15.1	18.8	16.3	4.63	5.91	5.25

* S calculated from cystine.

varies from 8.77 to 13.38 per cent with an average of 10.97 per cent and total nitrogen varies from 12.6 to 18.7 per cent with average of 15.8 per cent. The N : S ratio averages 5.40 and remains relatively constant with extremes of 4.61 and 5.92. The greater range of cystine in our group compared to that of Sullivan, using essentially the same method, may possibly be explained on the basis of variation in selection of individuals for the " normal " group.

Cystine content of finger nails in pellagra, syphilis, and pulmonary tuberculosis

The pellagrins are arranged into four arbitrary groups: Group I, severe pellagra with extensive dermatitis; Group II, severe pellagra without dermatitis; Group III, pellagra during recovery; and Group IV, completely healed pellagra. An exfoliative dermatitis of the hands, arms, and feet and in most cases of the legs and thighs had been apparent in the 14 cases in Group I for at least two months. All cases were extremely ill at the time of the determination and Cases 101, 108, and 115 (see Table II) died with inanition soon after the observation. A stomatitis was common to all and several had in addition diarrhea, psychosis, or dementia. In this group (see Figure 1, *C* and Table I) cystine is greatly reduced averaging 7.26 per cent. The highest result, 8.55 per cent, falls below the lower limit of the normal range. In Cases 120 and 127 (see Table I) in which

TABLE II

Cystine, total nitrogen, and N : S ratio in finger nails of individual negro cases of severe pellagra with extensive dermatitis before and after recovery

Case number	Sex	Age	Cystine	Total nitrogen	N : S ratio *	Clinical notes
		years	*per cent*	*per cent*		
101	F	50	7.04	16.0	8.51	Extensive dermatitis. Died 2 weeks later
107	F	60	7.46	15.8	7.94	Severe dermatitis. Later recovered
108	F	63	8.55	15.4	6.76	Severe dermatitis. Died 4 weeks later
109	F	53	7.52	12.6	6.27	Extensive dermatitis.
			11.80	15.7	4.98	4 months after recovery
111	F	32	7.69	13.8	6.74	Severe dermatitis. Later recovered
112	F	30	7.50	13.8	6.90	Extensive dermatitis.
			10.90	17.9	7.43	6 months after recovery
113	F	29	7.32	13.1	6.71	Severe dermatitis.
			8.22	16.3	7.41	3 months after recovery
114	F	46	7.27	12.6	6.50	Severe dermatitis.
			10.83	13.7	4.74	5 months after recovery
115	F	45	8.00	15.4	7.20	Extensive dermatitis. Died 9 weeks later
118	M	17	7.09	15.0	7.98	Severe dermatitis.
			10.72	14.3	5.00	6 months after recovery
119	M	70	14.49	19.2	4.95	3 months before onset of symptoms.
			7.85	14.5	6.90	Severe dermatitis
120	M	32	5.03	15.1	11.28	Most severe dermatitis.
			9.53	17.8	7.01	4 months after slow improvement
127	M	27	6.15	18.1	11.01	Very severe dermatitis. Later recovered
140	M	40	7.24	15.7	8.14	Severe dermatitis. Later recovered
				14 cases, severe dermatitis		
Minimum.....			5.03	12.6	6.27	
Maximum....			8.55	18.1	11.27	
Average.....			7.26	14.8	7.63	
				5 cases, following recovery		
Minimum.....			8.22	13.7	4.74	
Maximum....			11.08	17.9	7.43	
Average.....			10.49	15.6	5.91	

* S calculated from cystine.

dermatitis was most extensive and severe, cystine content is the lowest, 5.03 per cent and 6.15 per cent. A determination three months before the appearance of dermatitis and diarrhea in Case 119 (see Table I) is of particular interest since at that time the cystine content was 14.49 per cent. At this time, because of emaciation, the patient was taking a diet rich in vitamins and calories. Later he refused food and in three months developed a severe dermatitis. Six months after the original observation cystine had decreased to 7.85 per cent, a reduction of 46 per cent. In five cases results were obtained following recovery (see Table II). Cystine had returned to normal limits in all cases and at this time the average cystine, 10.49 per cent, was but slightly below the average for the normal group. Total nitrogen remained practically unchanged varying from 12.6 to 18.1 per cent with an average of 14.8 per cent. The reduction in cystine results in a relative increase in the N : S ratio.

Group II, severe pellagra without dermatitis, consists of six cases: four extremely emaciated, weak, demented individuals suffering from a persistent diarrhea of 15 to 25 stools a day for at least the past two months, and two cases with vicious dementia and mild diarrhea, weakness, and glossitis of seven and of thirty months duration. Although diarrhea was resistant to treatment, all cases recovered after a prolonged improved dietary régime. Skin lesions were observed in only one case and in this case the discoloration and fine desquamation over the feet were so greatly eclipsed by the violent dementia and diarrhea that he is included in this group. However, it is interesting to note that cystine in this case, 8.93 per cent, is the lowest value in the group. All values for cystine and total nitrogen (see Figure 1, *D* and Table I) fall within normal limits, cystine ranging from 8.93 to 11.98 per cent, and total nitrogen from 12.6 to 18.3 per cent. The average N : S ratio approximates that of the normal group. Determinations after recovery in two cases show no striking change in cystine nor in total nitrogen.

Group III consists of 10 cases all of whom had been recovering for the past two or three months from mild dermatitis of the feet and hands, and stomatitis. Intermittent diarrhea had been common and four cases had developed temporary psychoses. At the time of analyses dermatitis had disappeared and all were much improved. Cystine varied from 9.26 to 11.93 per cent, averaging 10.47 per cent, and total nitrogen 13.5 to 17.6 per cent, with an average of 15.5 per cent, all within normal limits (see Figure 1, *E* and Table I). The N : S ratio averaged 5.56 with extremes of 4.57 and 6.16. Results from three cases following complete recovery showed no change except in one case in which cystine increased from 9.37 per cent to 12.25 per cent, total nitrogen from 15.4 per cent to 16.9 per cent and the N : S ratio from 6.16 to 5.16.

There are 21 cases of healed pellagra in Group IV. In this group analyses were made 3 to 6 months after disappearance of all signs of pel-

lagra during which time the patient remained on an adequate diet containing an abundance of all vitamins. Thirteen had recovered from severe pellagra with extensive dermatitis, four from severe pellagra of the gastrointestinal or central nervous system type in which dermatitis had been absent or played a minor rôle, and four from mild diarrhea and slight dermatitis. In this group (see Figure 1, *F* and Table I) cystine varies from 8.22 to 15.42 per cent. Two results, 14.49 per cent and 15.42 per cent, lie above the upper normal limit. Total nitrogen varies from 13.6 to 19.8 per cent also showing a greater variation than normal in the upper limit. Both the average cystine and total nitrogen approximate the average of the normal group.

The group of 20 luetics consists of patients treated for at least two months at the syphilis dispensary of the Duke Hospital, and although at the time the analyses were made, none had active lesions, all had a history of luetic infection and the blood Wassermann test had been four positive. The group is representative of widely divergent dietaries as observed in mill-hands, farmers, shopkeepers, etc. in the pellagra belt. No definite deficiency could be ascribed to any diet although several of the patients were obviously malnourished and many consumed large amounts of corn and fat pork to the exclusion of fruits and other vegetables and meats. Cystine varies more widely in this group (see Figure 1 and Table I) than in the normal, 8.20 to 13.55 per cent, in three cases the result falling below the lower limit of normal, and in one case above the upper limit. However, the average for the group, 10.64 per cent, approximates the normal average. Total nitrogen tends to be increased varying from 14.1 to 21.9 per cent and averaging 16.9 per cent, thereby increasing the average N : S ratio to 5.95.

The 5 cases of pulmonary tuberculosis gave histories of loss of 20 to 60 pounds of weight during the preceding months and were obviously malnourished, judging by the severe emaciation. Cystine and total nitrogen tend to be above normal in these cases (see Figure 1, *G* and Table I), cystine ranging from 10.28 to 13.64 per cent and averaging 11.66 per cent, and total nitrogen from 15.1 to 18.8 per cent and averaging 16.3 per cent. Because of the concomitant rise of both cystine and total nitrogen the N : S ratio values fall within normal limits.

DISCUSSION AND SUMMARY

The foregoing results support the opinion that there is an abnormality of sulfur metabolism in pellagra which is reflected in epithelial tissue and specifically related to the dermatitis of this disease. A study of the data from 36 normals and 20 cases of syphilis shows no constant variation of cystine with difference in sex, race, or age. Since cystine was reduced and N : S ratio increased in the finger nails from all of the 14 cases of pellagra with severe dermatitis, whereas in all cases of pellagra without

dermatitis cystine and total nitrogen remained approximately normal, it is reasonable to assume that the reduction of cystine in finger nail is closely related to the pathological changes in the epidermal tissues in pellagra. This would seem to indicate that a lack of cystine or deficient utilization of this amino acid becomes manifest in the skin lesions of pellagra. On the other hand, such a deficiency of cystine cannot be held responsible for the entire symptom complex of pellagra, since cases of pellagra without dermatitis in whom there is no diminution of cystine in the epidermal tissue, as indicated by our data, are common.

Goldberger and Tanner (24) at one time advanced the theory of a deficiency of amino acids as the probable cause of pellagra and suggested that a deficiency in the supply or utilization of the amino acid, cystine, resulted in pellagra. Support for this theory in explaining the skin lesions of pellagra is given by the work of Harris and of Ward previously cited and by our data. Rough estimations of cystine in the dietary of our patients who developed pellagra failed to show a deficiency in the supply of cystine although a vitamin deficiency was probable. The high cystine values in the finger nails in the group following dietary treatment may indicate greater cystine utilization influenced by adequate vitamin consumption. However, as pointed out by Smith (25), the foods used in the treatment of pellagra contain large amounts of cystine and the increased concentration in healed cases may be due to increased intake of this amino acid *per se,* especially since Beadles et al. (26) have demonstrated that the rat's coat of hair becomes heavier when the diet is supplemented with cystine.

Injections of thiosulfate have been advocated and extensively used with apparent benefit in the treatment of pellagra. Sabry (27) reports that the skin lesions of pellagra disappear after a few injections, and that there is marked improvement of diarrhea and of the central nervous system manifestations of this disease after a short course of treatment, consisting of daily intravenous 10 cc. injections of 10 per cent solution of sodium thiosulfate. Although Goldberger et al. (19, 20) have demonstrated that pellagra may be prevented and cured by supplying the pellagra-preventing factor (vitamin G) in the diet of the pellagrin, and although the thiosulfate treatment is usually combined with an improved dietary régime, in view of the demonstrated alteration in sulfur metabolism, it is likely that thiosulfate is of some benefit to the pellagrin and that some of the rapid improvements observed may be due to this factor directly.

Although we cannot conclude that because of a reduction of cystine in finger nails there must be a reduction of cystine in the skin and therefore an increased sensitiveness to light, it is reasonable to suspect that a reduction of nail cystine is accompanied by a reduction of skin cystine. We contemplate further experimental work to determine the importance of this change in sulfur metabolism and its relation to solar radiation in pellagra.

CONCLUSIONS

1. The cystine content of finger nails was determined in 36 normal subjects, in 40 cases of various forms and stages of pellagra, and in 25 cases of syphilis and tuberculosis.

2. In 14 cases of pellagra with extensive dermatitis there was a marked reduction in the cystine content without any appreciable change in the total protein content of the finger nails. With the subsidence of skin symptoms and improvement in the clinical condition, the cystine content returned to normal limits.

3. In severe pellagrins without dermatitis (involvement chiefly of the gastro-intestinal or central nervous systems) and in partially or completely cured cases of pellagra there were no marked changes in the cystine content of the finger nails.

4. Values for cystine content of the finger nails from a group of syphilitics and from a group of tuberculous patients were found to approximate the normal range regardless of the nutritional state of the patient.

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