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Identification of Heterozygous Carriers of Gargoylism. (26913)

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In recent years gargoylism (Hurler's syndrome, dysostosis multiplex) has been found to be a hereditary disorder of acid mucopolysaccharide (AMPS) metabolism(1-3). Thorough evaluation of pedigrees of affected families has indicated 2 forms of gargoylism: the autosomal recessive and the sex-linked recessive types(4). Many patients with overt gargoylism can be identified by an elevated urinary level of total AMPS(2-4) and by the presence of abnormal acid mucopolysaccharides such as chondroitin sulfate B(2-4) (ChS-B) and heparin monosulfate(3,5) (HMS) in their urine. Up to the present time, however, it has not been possible to identify chemically the heterozygous carriers of the gargoylism disease trait.

For the characterization of acid mucopolysaccharides, Hoffman and co-workers(6) have used the hexuronic acid carbazole/orcinol (C/O) color extinction ratio. In an analogous manner we have used the carbazole (7)/naphthoresorcinol(8) (C/N) color ratio. We obtained a C/N color ratio of 13.6 for chondroitin sulfate A* (ChS-A), 0.6 for chondroitin sulfate B*, 14.0 for chondroitin sulfate C* (ChS-C), and 12.2 for heparin monosulfate†. The C/O color ratios reported by Hoffman and co-workers(6) for ChS-A and ChS-C are only about 4 times the C/O ratio for ChS-B whereas the C/N color ratios we obtained for ChS-A and ChS-C are about 20 times the C/N ratio for ChS-B. A low C/N ratio appears to be characteristic of ChS-B.

The usefulness of the AMPS C/N ratio was discovered in our search for better methods of detecting the ChS-B and the elevated level of total AMPS in the urine of patients with clinical gargoylism. In some cases a simple quantitative index of the AMPS in urine was found to be inadequate for chemical verification of an abnormal AMPS excretion. Addition of the C/N ratio as an index of urine AMPS quality to the index of AMPS quantity has proved to be much more specific than the quantitative index alone. Our results indicate that patients with suspected gargoylism as well as their normal parents and other relatives with the heterozygous trait of gargoylism can now be identified.

Our method, performed on a single urine specimen, involves preliminary dialysis to remove most of the smaller uronic acid-containing molecules; concentration of the AMPS by precipitation with 5% cetyltrimethyl-ammonium bromide according to the DiFerrante and Rich(9) procedure; and determination of the AMPS hexuronic acid by the carbazole method of Dische(7) and the naphthoresorcinol method of Pelzer and Staib(8). The quantity and quality of the AMPS is then represented respectively as a

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TABLE I. Urinary Excretion of Acid Mucopolysaccharides by Normal Persons, Patients with Gargoylism, and Heterozygous Carriers.

		Mean ± S.E.*	S.D.†	P‡
Normal persons (all ages)	C/Cr§	5.1 ± .7	4.1	
	C/N‖	15.1 ± .9	5.6	
Patients	C/Cr	53.8 ±7.1	25.7	<.01
	C/N	4.2 ± .3	1.2	<.01
Suspected heterozygous carriers	C/Cr	3.6 ± .6	3.1	>.1
	C/N	4.5 ± .3	1.5	<.01

* Mean value ± stand. error of mean.
† Stand. dev.
‡ P value for comparison with normal excretion.

§ C/Cr ratio $= \frac{\text{mg of carbazole-hexuronic acid}}{\text{g creatinine}}$

‖ C/N ratio $= \frac{\text{mg of carbazole-hexuronic acid}}{\text{mg of naphthoresorcinol-hexuronic acid}}$.

carbazole/creatinine (C/Cr) ratio $\left(\frac{\text{mg of carbazole-hexuronic acid}}{\text{g of creatinine before dialysis}}\right)$ and a carbazole/naphthoresorcinol (C/N) ratio $\left(\frac{\text{mg of carbazole-hexuronic acid}}{\text{mg of naphthoresorcinol-hexuronic acid}}\right)$.

The mean C/Cr ratio and C/N ratio of the AMPS excreted by 38 normal human subjects of both sexes and all ages, by 13 patients with gargoylism, and by 27 suspected heterozygous carriers of gargoylism are compiled in Table I. Both the mean C/Cr ratio and the C/N ratio of the patients were significantly different from normal values, the C/Cr ratio being much higher and the C/N ratio lower than normal. The suspected heterozygous carriers revealed a normal mean C/Cr ratio but a C/N ratio which was significantly lower than the normal mean C/N ratio and of the same order as the C/N ratios in patients with overt manifestations of gargoylism. The low C/N ratios suggest that a certain proportion of the assayed AMPS material consists of ChS-B. Since ChS-B is not normally excreted in the urine(2-4), the occurrence of low C/N ratios in some clinically healthy relatives of patients with gargoylism most likely indicates the heterozygous carrier state of the disease trait. Actual isolation and identification, however, of ChS-B from these urines has not been performed.

C/N ratios obtained from 10 patients with gargoylism and their respective parents are shown in Table II. In 7 instances both father and mother revealed low C/N ratios, suggesting an autosomal recessive mode of inheritance. In one family the father had a normal C/N ratio which would indicate the sex-linked recessive type of transmission.

We did not find significant difference in the nature of AMPS excreted by patients with the autosomal recessive and the sex-linked recessive form of gargoylism. Also, we have not had an opportunity to examine the urinary AMPS of a patient who excreted only HMS as described by Harris(5).

Summary. The carbazole and naphthoresorcinol methods for hexuronic acid deter-

TABLE II. Urinary Acid Mucopolysaccharides Excreted by Patients with Gargoylism and Their Parents.

Case	Sex	C/N ratios* Patient	Father	Mother	Suggested mode of inheritance
1	♂	6.0	10.0	4.1	Sex-linked recessive
2	♀	3.3	5.0	2.3	Autosomal recessive
3	♀	3.6			
4	♂	2.6	3.3	4.0	" "
5	♂	4.2	3.9	4.6	" "
6	♂	5.1	3.4	4.7	" "
7	♂	5.0	4.5	2.7	" "
8	♂	3.7	7.5	5.9	" "
9	♂	4.8			
10	♂	5.0	6.4	5.9	" "

* C/N ratio $= \frac{\text{mg of carbazole-hexuronic acid}}{\text{mg of naphthoresorcinol-hexuronic acid}}$ (normal range: 15.1 ± 5.6).

mination have been used to characterize the partially purified acid mucopolysaccharides obtained from urine of normal persons, from patients with gargoylism and from their relatives. The results, expressed as a ratio of carbazole to naphthoresorcinol hexuronic acid, demonstrated a similar abnormal qualitative excretion of chondroitin sulfate B by patients with gargoylism, by one or both parents, and by some of their other relatives. Occurrence of an abnormal qualitative excretion of chondroitin sulfate B in the clinically normal relatives of patients with gargoylism probably indicates the heterozygous carrier state of the disease.

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Rate of Fat Uptake by Intestinal Lymphatics.* (26914)

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The time course of fat absorption has been studied most frequently by following the changes in blood lipid concentration at intervals after fat meals using either the chylomicrograph(1,2) or chemical methods(3) for analysis of plasma lipids. Disappearance of the excess lipid from the circulation after fat feeding has often been taken as an index of the completion of fat absorption, but this is based upon at least 2 important assumptions: 1) that the methods of measurement are sufficiently sensitive to detect extremely small amounts of absorbed lipid and 2) that the rate at which fat is added to the circulation during fat absorption always exceeds the rate at which it is removed. The time at which the excess lipid disappears following a fat meal is highly variable, ranging from 4 hours (1) to 10 hours(2) using either of the above methods. However, recent studies using the more sensitive I^{131}-labelled triolein technic have demonstrated the continued presence of I^{131}-labelled lipid in the circulation for periods extending beyond 24 hours after its oral administration(4) suggesting either a delayed removal rate or a prolonged absorption period. In the present study evidence will be presented to show that absorbed fat may continue to enter the circulation through the thoracic duct for 24 hours or more after a single oral fat load. Some of the reasons for this prolonged absorption period are examined and discussed in relation to the above mentioned studies involving the simple measurement of plasma lipid concentration during alimentary lipemia.

Methods. The study was carried out in dogs ranging in size from 10 to 18 kg, maintained on a nutritionally adequate "fat-free" diet for a variable period of time prior to experiment. This diet contained: purified casein, 24%; sucrose, 40%; corn starch, 16%; cellulose, 14%; salt mixture (U.S.P. IV), 4%; complete vitamin mixture, 2%;

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