disposed, it is easy to see that the amount of specifically precipitated nucleotidic material is proportional to the number of strands involved in the reaction. With this observation in view, the number of strands in a molecule can be determined immunochemically if reference values corresponding to an unordered single-stranded molecule are available.

Finally, the organization of polyribonucleotides determines the degree of masking of antigenic sites. The antigenic sites are probably located along the polyribosephosphate backbone. This statement is supported by the facts that, on the one hand, the nature of the base is not a quantitatively determining factor in the reaction, and, on the other hand, the NG-I antibodies can still precipitate two-, three-, and even four-stranded molecules in which the bases are hidden in the interior of the molecule. The establishment of a helical structure would create, at regular intervals, a steric hindrance to the approach of antibodies, and cause masking of antigenic sites which results in a decrease in the amount of antibody precipitated.

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Beta-Glucuronidase Activity in Serum Increased by Coronary-Artery Atherosclerosis

Abstract. *Increase in activity of beta-glucuronidase in serum has been demonstrated in patients having clinically evident coronary-artery atherosclerosis. This fact, yielded by the new, more specific method of Fishman, could not be elicited by the traditional method.*

Use of Fishman's new and more specific method for the determination of beta-glucuronidase activity in serum (*1*) has enabled demonstration of markedly increased activity in patients having coronary-artery atherosclerosis. The new method employs sixfold increase of the phenolphthaleinglucuronide substrate and reduces the time of incubation from 20 to 4 hours; it gives values roughly twice as high as those obtained by his previous method (*2*), and the new values correspond closely with values obtained from serum from which inhibitors have been removed by dialysis.

Our study dealt with 49 patients of whom each had either classical symptoms of angina pectoris or a documented history of coronary-artery occlusion with myocardial infarction (in patients in whom the clinical episode had occurred months or years previously, so that one may assume that their increased beta-glucuronidase activities in serum did not result from ischemic necrosis of cardiac muscle). None of the patients showed clinical or laboratory evidence of diabetes mellitus, liver disease, or pregnancy, any of which increases beta-glucuronidase activity.

The controls selected were patients either attending the hospital diagnostic clinic or awaiting elective surgery such as herniorrhaphy; all were free of clinically apparent cardiovascular diseases or other overt illness, and matched the coronary-atherosclerosis patients as closely as possible as to age, sex, and race.

The 49 test patients showed an average activity of 2140 units, versus 1270 units for the 67 controls ($P < .001$). Eighty-five percent of the controls gave values below 2000 units, whereas 58 percent of the test patients ranged above 2000 units; only one control exceeded 2500 units, whereas 37 percent of the test patients ranged between 2500 and 5310 units. When the results for the two groups were compared by age, sex, or race, activity was always significantly higher for the coronary patients than for the controls (*3*).

Others (*4*) were unable to demonstrate increased activity in patients having coronary-artery disease by the traditional method of Fishman, Springer, and Brunetti (*2*), which we also used on each of the specimens of serum studied by the new Fishman procedure. The less specific method showed no significant differences in mean enzyme activity between the test patients and the controls. A fluorometric procedure has shown a suggestive increase in female but not in male patients (*5*).

Several workers using the less specific method have found increased

activity in patients having diabetes mellitus but we could not confirm the finding. However, using the specific method we have demonstrated highly significantly increased activity in 100 diabetic patients (*6*). It is well known that patients having diabetes mellitus have greater tendency to develop coronary-artery and other types of atherosclerosis than do nondiabetics. Thus it is tempting to speculate that the increased activity may point to a common biochemical pathway for the genesis of atherosclerosis in nondiabetics and in patients having diabetes mellitus; such a pathway may be the glucuronic acid cycle. Winegrad *et al.* postulated that this insensitive-to-insulin cycle is hyperactive in patients having diabetes mellitus, because they demonstrated increased serum levels of L-xylulose, a component of the glucuronic acid cycle (*7*). Accelerated activity of the glucuronic acid cycle would presumably produce more uridine diphosphate glucuronic acid and in this way stimulate synthesis of mucopolysaccharides (*8*). Because beta-glucuronidase participates in the degradation in vitro of mucopolysaccharides (*9*), accumulation of excess amounts of mucopolysaccharides may secondarily induce greater beta-glucuronidase activity in order to restore the mucopolysaccharides to more normal levels.

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14 February 1966

Homocystinuria: Excretion of a New Sulfur-Containing Amino Acid in Urine

Abstract. *An unusual homocysteine-containing compound, 5-amino-4-imidazolecarboxamide-5′-*S*-homocysteinylriboside, was isolated from the urine of a child with homocystinuria and detected in the urines of six homocystinurics. Its metabolic origin is not clear, and the data suggest the existence in man of now unknown alternate pathways for the metabolism of methionine and of purines.*

Homocystinuria is an inborn error of methionine metabolism and is characterized clinically by mental deficiency, dislocated ocular lenses, and skeletal abnormalities, and by the occurrence of serious arterial and venous thromboses (*1*). Patients have elevated concentrations of methionine and homocystine in blood, and homocystine is excreted in the urine. The fundamental biochemical defect in the disease is a deficiency of the enzyme cystathionine synthase (*2*), with the normal formation of cystathionine from homocysteine and serine failing to take place both in liver and brain cells. Cystathionine, one of the major free amino acids of human brain, is absent or markedly diminished in the brain tissue of patients who have died with homocystinuria (*3*).

In homocystinuria the urinary excretion of homocystine and methionine accounts for only a small fraction of the daily dietary intake of methionine, even though the major degradative pathway in methionine metabolism is blocked at the step of cystathionine synthesis (*4*). While searching for additional sulfur-containing compounds in urine to explain this discrepancy, we found an unidentified ninhydrin-positive spot which was routinely present on two-dimensional amino acid paper chromatograms prepared from the urines of homocystinurics (Fig. 1), but which we never encountered in the urines of normal individuals.

The unknown compound migrated to a locus close to, but distinct from, that occupied by histidine when urines were chromatographed first in a mixture of pyridine, acetone, ammonium hydroxide, and water (45 : 30 : 5 : 20), and then in a mixture of isopropanol, formic acid, and water (75 : 12.5 : 12.5). Its positive reaction to the chlorplatinate spray (*5*) indicated that it contained unoxidized sulfur. Its failure to react to sodium nitroprusside alone, or to nitroprusside after prior exposure either to sodium cyanide or to methanolic NaOH, indicated that the unknown compound did not contain a sulfhydryl, disulfide, or thioester group, but was consistent with its being a thioether. The unknown compound also strongly absorbed ultraviolet light when chromatographed on paper in solvent systems not containing pyridine.

Several milligrams of the unidentified compound were isolated from a homocystinuric child's urine by ion-exchange column chromatography. A portion of urine containing 100 mg of creatinine was concentrated to a small volume by lyophilization and applied to the surface of a column (45 by 2.16 cm) of Dowex 50 × 8, buffered with pyridine acetate at *p*H 3.50 (0.1*M* in pyridine). The same buffer was then pumped through the resin column at 50°C at a flow rate of 50 ml/hr. The unknown sulfur-containing compound was eluted between 730 and 790 ml. The volatile buffer was removed from this effluent zone under reduced pressure on a rotary evaporator, and the compound was further purified by chromatography on Whatman No. 3MM paper, first in a mixture of pyridine, acetone, ammonium hydroxide, and water (45 : 30 : 5 : 20), and then in a mixture of isopropanol, formic acid, and water (75 : 12.5 : 12.5).

The isolated compound has now been tentatively identified as 5-amino-4-imidazolecarboxamide-5′-*S*-homocysteinylriboside (AICHR) (Fig. 2).

When the purified compound isolated from urine was chromatographed on the short column of the Technicon amino acid analyzer, with the buffer gradient system for protein hydrolyzate analysis (*6*), it was eluted as a single peak at the same effluent volume as ammonia and homocystine. When applied to the long column (127 cm) on the analyzer, and with the buffer gradient system recommended by Efron (*7*), the compound was eluted as a single peak 17 minutes after homocystine and 2 hours and 55 minutes before ammonia.

Repeated hydrolyses of the urinary compound in 5.7*N* HCl at 110°C for 16 hours yielded homocystine, homocys-