factor." The following information offers proof of this.

The assay of myoglobin and other materials for growth activity in *C. briggsae* is similar to that reported (*3, 7*). The chemically defined medium employed was originally reported by Sayre *et al.* (*2*) and modified by Buecher *et al.* (*10*). It consists of pure amino acids, glucose, water soluble vitamins, salts (including iron), and ribonucleotides (*11*). This mixture was further supplemented with sterols (50 μg/ml) in Tween 80 as described (*7*), and with autoclaved *E. coli* cells [0.3 mg/ml (dry weight)], which altogether comprise the basal medium. Axenic larvae of *C. briggsae* (usually three) were inoculated into a 10- by 75-mm culture tube that contained 0.25 ml of medium (in duplicate) and were incubated at 20°C. The effectiveness of the medium was determined by observing at intervals of 1 day or less the growth in length of larvae and reproduction of adult individuals.

In the basal medium alone, larvae grow to the size of advanced fourth stage larvae or small adults (650 to 850 μm long) in about 4 days, but they do not reproduce. As little as 0.31 percent (by volume) of heated liver extract (equivalent to 1.6 mg of original liver per milliliter of medium) supported extensive reproduction of the larvae (Table 1). Doubling the concentration of heated liver extract had little effect, whereas 0.16 percent proved to be inadequate.

As long as autoclaved bacterial cells are added, purified myoglobin (equine heart, Pierce Biochemicals, Rockford, Ill.) can replace heated liver extract as a growth requirement (Table 1). Myoglobin was dissolved in water at a concentration of 5 mg/ml, sterilized by membrane filtration (0.45 μm pore size, Millipore Filter Corp., Bedford, Mass.) and added to the above basal medium to give concentrations of myoglobin from 12.5 to 100 μg/ml.

Myoglobin becomes more effective for growth after autoclaving, although it does not effectively support growth at 12.5 μg/ml. The crude liver extract, at concentrations that support substantial growth, contained only 10 μg of hemeproteins per milliliter. However, autoclaved myoglobin at 100 μg/ml supports rapid reproduction and repeated subculture (ten serial transfers) of *C. briggsae* when it is substituted for heated liver extract.

A number of other proteins were tested in the same basal medium. Hemoglobin and cytochrome c were slightly more effective than myoglobin on a weight basis. Both compounds retained their activity after autoclaving. Non-hemeproteins such as β-lactoglobulin, bovine serum albumin, and soluble casein, each at a concentration of 100 μg/ml, were inactive (Table 1). The nutritional requirement for reproduction of *Caenorhabditis briggsae* under these conditions thus seems to be satisfied generally by hemeproteins, but not by protein in the absence of heme.

A question remains as to whether the active factor is the Fe-porphyrin moiety of the hemeprotein complex. A partial answer was obtained from experiments with hemin. Hemin chloride (Calbiochem, Los Angeles, Calif.) was dissolved in 0.1*N* potassium hydroxide, neutralized with hydrochloric acid to *p*H 7.8, diluted to 1 mg/ml, and sterilized by Millipore filtration in the same way as the myoglobin solution (Table 1).

At 8 μg/ml (equivalent to 200 μg of myoglobin on a molar basis), hemin was as effective as myoglobin at 100 μg/ml (Table 1); at 4 μg/ml hemin was approximately as effective as myoglobin at 50 μg/ml. At 2 μg of hemin per milliliter the generation time increased significantly and the total population was small. The 1 μg/ml concentration was ineffective. Therefore, on a molar basis, hemin is only about one-half as active as myoglobin although it is an effective substitute. One may speculate that the differences in effective doses could be related to problems of absorption; for example, precipitated versus soluble forms.

The results clearly demonstrate that hemin or heme compounds are required for reproduction of *C. briggsae* in a defined medium supplemented with sterols and autoclaved *E. coli* cells. Thus, one of the active components of liver extract (or of fractions derived from it) is most probably a hemeprotein. The meaning of the requirement for specialized structures reported by Sayre *et al.* (*5*) for their growth factor is unclear, especially since the material is replaceable by three contituents—a heme compound, a sterol, and a heat-stable component present in bacterial cells.

The requirement for heme may be related to the metabolism of hemeproteins in the parasitic nematode *Ascaris lumbricoides*. Smith and Lee (*12*) reported that the hemoglobin content of perienteric fluid increased when hemeproteins or porphyrins were added to the incubation medium; much hematin was incorporated into developing eggs of this organism.

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Pepsinogens: Genetic Polymorphism in Man

Abstract. *Population frequencies of the two patterns of pepsinogen excretion (with and without pepsinogen 5) and family studies indicate that pepsinogen 5 is controlled by a pair of genes,* Pg^a *and* Pg^b, *at a single autosomal locus and that* Pg^a *is dominant.*

We have demonstrated the existence of seven electrophoretically distinct pepsinogens (Pg 1–Pg 7) in extracts of human gastric mucosa, and have shown that they are clearly separable into two groups (*1, 2*). The five faster migrating zymogens, Pg 1–Pg 5, are limited to the fundus and body of the stomach and have been termed the group I pepsinogens. Pepsinogens 6 and 7 are also found in the antrum and proximal duodenum and have been designated the group II pepsinogens (*1, 2*). With the exception of Pg 1, the

group I pepsinogens are found in urine, whereas the group II pepsinogens are rarely found in normal urine (*2*).

Some individuals lack Pg 5 in their gastric mucosa, and such individuals fail to excrete this zymogen in their urine. Subjects with Pg 5 in their mucosa also excrete this zymogen in their urine. Pepsinogens 2, 3, and 4 are invariant members of this group, having been found in the urine or gastric mucosa of every subject studied. The two patterns of pepsinogen excretion have been designated A (with Pg 5) and B (without Pg 5) (Fig. 1). Each pattern is constant for the individual (*2*).

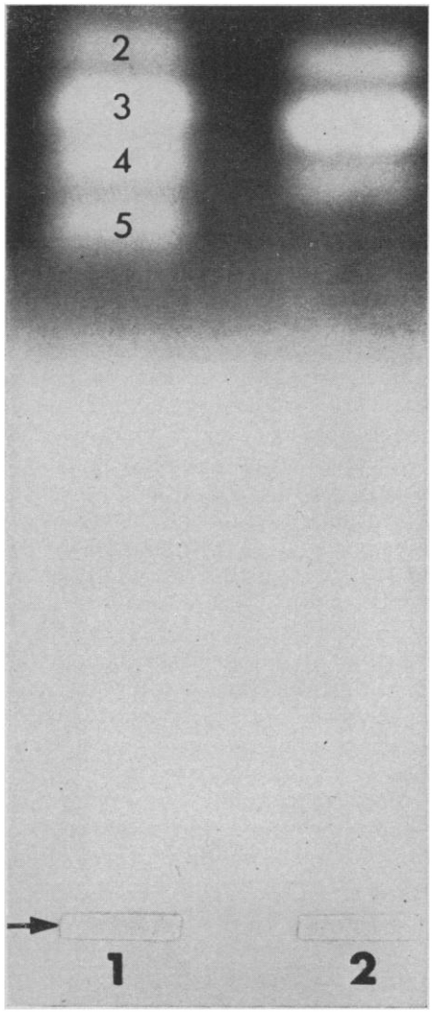

Fig. 1. Zymogram of pepsinogens in two urine specimens demonstrating pattern A (slot 1) and pattern B (slot 2). Pepsinogen 5 (Pg 5) is absent in pattern B. The specimens were subjected to electrophoresis in 1.5 percent agar in 0.05*M* barbital buffer (*p*H 8.3) for 3.5 hours at 11 volt/cm. The origin (arrow) is at the cathodal end of the plate. Proteolytic activity was demonstrated by immersing the plate in 0.65 percent bovine hemoglobin (*p*H 1.4) for 10 minutes after which it was incubated in a humid chamber for 1 hour at 37°C. The plate was then placed in a solution of 5 percent acetic acid in 50 percent ethanol to fix undigested protein, dried, and stained with amido black.

This study demonstrates that the presence or absence of Pg 5 is genetically determined. To determine the frequency of each pattern, urine was obtained from 931 unrelated Caucasian individuals without known peptic ulcer disease. There were 405 males and 526 females ranging in age from 2 days to 80 years. The molecular forms of pepsinogen in each subject's urine were determined by electrophoresis in agar gel (*1*, *2*).

Of the 931 subjects 799 (85.82 percent) had pattern A and 132 (14.18 percent) had pattern B. There was no association of pepsinogen pattern with sex (Table 1).

The possible genetic determination of patterns A and B has been studied in 100 matings involving 75 unrelated families (Table 2). Of 148 progeny of 58 A × A matings, 137 were A and 11 were B. Of 92 offspring of 33 A × B matings, 65 were A and 27 were B. Each of the six progeny of three B × B matings was B.

The proposal which best explains these data is that Pg 5 is controlled by a single pair of autosomal allelic genes, Pg^a and Pg^b, with Pg^a being dominant. Thus, individuals with phenotype A may be either homozygotes (Pg^a Pg^a) or heterozygotes (Pg^a Pg^b). The frequency of each gene, as estimated from the results of the population study with the Hardy-Weinberg law is 0.6235 for Pg^a and 0.3765 for Pg^b. Based upon these data, the expected frequency of the phenotypes of the progeny resulting from the matings can be calculated (Table 2). The predicted values closely fit the observed data. We conclude that the absence of Pg 5 is inherited as a simple autosomal recessive trait.

The localization of the group I pepsinogens to the fundus and body of the stomach suggests that these zymogens are synthesized by the chief cells. A genetic polymorphism has here been demonstrated for Pg 5, but the importance of this locus in the genetic determination of the invariant members of the group (Pg 2–Pg 4) is not known. Conceivably, one or even all of the invariant members may be determined by the same locus, with the polymorphism reflected only in Pg 5. On the other hand, another locus or other loci may determine the invariant members. The constancy of these invariant members precludes any conclusions concerning their relationship to Pg 5 or to the number of loci involved in their determination. The observed heterogeneity does not, however, result from polymerization since Pg 2–Pg 5 are eluted together from the included volume of Sephadex G-75 (*1*). This observation lends support to the interpretation that another locus or other loci are involved in the determination of the invariant members.

Family studies and investigations of the relationship of blood groups to peptic ulcer disease and to pernicious anemia have shown a genetic predisposition to these disorders (*3*). The genetic polymorphism of Pg 5 may relate to the pathogenesis of these disorders.

Table 1. Frequency of pepsinogen patterns A and B among 931 subjects (405 males and 526 females). Numbers in parentheses indicate percentages in each category. The slight differences in frequency between males and females are not significant ($\chi^2 = 1.80$; $P > .10$).

Pepsinogen pattern	Total group	Sex	
		Males	Females
A	799 (85.82)	340 (83.95)	459 (87.26)
B	132 (14.18)	65 (16.05)	67 (12.74)

Table 2. Parental mating phenotypes and distribution of A and B phenotypes among offspring. Figures in parentheses are those expected if Pg 5 is controlled by two alleles (Pg^a and Pg^b) at a single autosomal locus and Pg^a is dominant.

Mating phenotypes	Matings (No.)	Offspring phenotypes (No.)		
		A	B	
A×A	64	137 (136.9)	11 (11.1)	$\chi^2 < 0.01$; $P > .95$
A×B	33	65 (66.8)	27 (25.2)	$\chi^2 = 0.177$; $P > .65$
B×B	3	0 (0)	6 (6)	

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