EFFECT OF HIGH MEAT DIET ON BACTERIAL FLORA AND CHEMICAL COMPONENTS OF HUMAN FECES: A PRELIMINARY REPORT*

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Introduction

As the title indicates, our project deals with the influence of a high meat diet on the bacteriological and chemical composition of the feces of humans. We are determining both qualitative and quantitative changes that occur in the intestinal tract as a result of high meat intake.

Recently, there has been interest in the relationship of diet to colon cancer. Several observations suggest that the incidence of cancer of the colon in a population may be related to diet. Epidemiological studies of areas with populations at high risk for colon cancer and areas with populations at low risk for colon cancer have shown that the most striking difference in these populations is the food they eat. The incidence of colon cancer is much higher in industrialized countries, in northwest Europe and North America, where a great deal of animal fat and protein and refined carbohydrates are consumed, than it is in the developing countries of Africa and South America and in rural India and Japan where much less meat is consumed and the diet is high in vegetable fiber (1).

That these variations in incidence are not simply related to geographical or genetic differences has been borne out by a number of observations. Migrant groups tend to assume colon cancer incidence rates of their adopted countries (2). In Japan, for example, the death rate for large bowel cancer is relatively low. In a study of mortality among U.S. Japanese in 1968, the migrants to the U.S. showed an increased incidence approaching that of the U.S. population (3). The migrant Japanese retained their low incidence status, however, provided they did not change their eating habits. The process of "westernization," either in California or Japan, appears to be associated with a high incidence of colon cancer.

All of these data led to the suggestion that the high animal protein and fat diets, characteristic of high risk populations, are responsible for colon cancer. Gregor and his associates (4) in a study of 33 countries found strong positive correlations between the incidence of colon cancer in a country and the amount of meat the population consumed. The high

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incidence has been attributed to the nature of the intestinal flora which synthesizes carcinogenic agents from the food and intestinal secretions, such as bile acids (5). Diet not only influences the composition of the intestinal flora, but also the quantity of substrates available for the production of carcinogens.

With this in mind, Hill and others (6) examined the fecal flora of individuals from different parts of the world. Fecal samples were obtained from individuals from six areas--England, Scotland and the United States, with a high incidence of colon cancer and Uganda, India, Japan with a low incidence of colon cancer. The same broad groups of bacteria were found in feces from all the populations studied. There were, however, substantial differences in relative numbers for several of the bacterial groups. The British and American subjects yielded many more gram-negative nonsporing anaerobes (Bacteriodes spp.) than did the Ugandans, Indians, or Japanese. Conversely, the Ugandans, Indians, and Japanese had many more aerobic bacteria (streptococci and enterobacteria), so that the ratio of anaerobes to aerobes was much higher in the people living on a western diet than in those on the largely vegetarian diets.

In an attempt to elucidate the factors leading to these differences, feces were examined from a small group of English people living in Uganda on a normal western diet. (6) These subjects had a fecal flora virtually the same as that of English people living in England on a mixed diet. Further, black Americans living in Atlanta had a fecal bacterial flora qualitatively and quantitatively the same as white Americans in Atlanta. These results suggest that neither race nor climate is a major determinant of the fecal bacterial flora, but that diet affects its composition.

What are some of the effects of consuming a western type diet high in animal fat and protein and low in fiber? Hill and others (6) found that the concentrations of acid and neutral steriods differed significantly in the feces of individuals on high meat and meatless diets. Feces collected from British and Americans on high meat diets contained much higher concentrations of steriods than feces of Ugandans, Indians, and Japanese whose diets contained little or no animal fats and proteins.

The neutral steriod concentration was very low in the feces of Ugandans and Indians, intermediate in the feces of Japanese and high in the feces of British and Americans. In addition, the microbial degredation products of cholesterol, coprostanol and coprostanone, constituted a much smaller proportion of the total neutral steriods in the feces of the Ugandans, Indians and Japanese than in the feces of the western group. This suggested that the bacterial flora of those individuals on a high meat diet is much more active in degrading cholesterol than the flora of those on an essentially vegetarian diet.

Even more striking differences were noted in the fecal concentration of acid steriods between the Ugandans, Indians and Japanese and the western group. The acid steriod concentrations were approximately eleven times greater in the feces of British and Americans than in the feces of Ugandans and Indians and approximately seven times greater

than in the feces of Japanese. Moreover, the extent of degradation of the acid steriods was higher in the British and Americans than in the other groups. Dehydroxylation reactions by intestinal bacteria resulted in monosubstituted and unsubstituted cholonates which were present in high proportions in the feces of the British and Americans. This is significant because the structure of bile acids is similar to that of known carcinogenic agents. Chemically, the naturally occurring bile acid, deoxycholic acid, has been converted into the potent carcinogen, 20-methyl-cholanthrene. It is noteworthy that the dehydroxylation reactions occurred more commonly among strains of bacteria isolated from feces of the English than from strains isolated from the feces of Ugandans.

As a result of all of these observations a theory was proposed that the typical diet of western peoples, which is high in animal fat and protein, increases the amount of bile secreted into the bowel. The diet also supports a microbial flora that is capable of converting the acid steroids into carcinogenic agents.

In their continuing investigation of the etiology of colon cancer, the Colon Cancer Segment of the National Cancer Institute felt it was essential, at this point, to check out this theory and to do a systematic study of the effect of a high meat diet on the bacterial and chemical composition of the feces of humans. They proposed a study, the subject of this report, in which the feces of individuals, maintained on meatless and subsequent high meat diets are monitored for changes in flora and chemical composition. The hope is that this information will provide an insight into the role intestinal bacteria play in converting food and intestinal secretions into active carcinogenic substances.

Methods

Since this represents a relatively extensive study, it was essential to obtain the cooperation of a number of investigators in several different laboratories within the University of Missouri. We were fortunate to locate ten male graduate and medical student volunteers who undertook a four month diet series. The series consisted of four sequential, one month diets including a normal diet, a meatless diet, a high meat diet, and again a normal diet. The diets were carefully formulated to standardize calories, proteins, fat, carbohydrates, cholesterol, fiber, minerals, and vitamins. It is significant that the fat and fiber contents were held constant in all four diets. Total protein was the same in the normal and meatless diets, but doubled during the high meat diet. Meals were prepared and served exclusively in the metabolic kitchen in the University of Missouri Medical Center. Chemical analyses for each of the diets were prepared on composite food samples collected during a one day period. Analyses were made of proximates, minerals, fatty acids, and total individual amino acids.

During the fourth weeks of each diet, three fecal samples were collected from each of the volunteers. Samples were collected in Gas-Pak jars set into a specially designed commode. The commode was constructed so that a mild stream of N_2 gas purged the collection jar during fecal passage thereby excluding oxygen and protecting oxygen sensitive organisms in the feces.

At the bacteriology laboratories, the anaerobic jars containing the specimens were placed into clear plastic oxygen-free glovebox isolators for processing anaerobic bacteria (7). Moisture determinations were made on all specimens and serial 10-fold dilutions of the specimens were prepared and plated on pre-reduced media. The colonies were counted and approximately 35-40 different representative colonies were picked from the anaerobic plates and identified. Species from among the genera Bacteroides, Eubacterium, Propionibacterium, Bifidobacterium, Fusobacterium, Clostridium, Peptostreptococcus, Actinomyces, and Veillonella were encountered.

The facultative laboratory received the 10-fold dilution series from the anaerobe laboratory. These dilutions were plated and colonies were picked and identified. In this case, we were dealing with species of coliforms, streptococci, lactobacilli, Bacillus, staphylococci, filamentous fungi and yeasts. From each stool specimen, a total of approximately 70 colonies were identified. Recovery, expressed as percent of total microscopic counts, was between 60 and 70%.

After a portion of the specimen was processed by the bacteriology laboratories, the remainder of the specimen was weighed, anaerobically sealed, and frozen for later delivery to the chemists. Three specimens collected from an individual during the last week on each diet were pooled and homogenized in 2 volumes of water under nitrogen. The homogenate was then distributed to the various chemistry laboratories. The feces were analyzed for the major classes of compounds listed in table 1. Determinations of many of the compounds involved considerable preliminary work in the development of new techniques or at least modifications of existing techniques. For example, techniques had been worked out for analysis of tryptophan metabolites in urine, but not for similar analysis with feces. New methods were developed for detection of ten potentially carcinogenic tryptophan metabolites from fecal material. In addition, techniques were developed for creatine and creatinine analyses, and for the determination of acid and neutral steroids in feces. All data from completed analyses were subjected to statistical evaluation.

Table 1. Chemical composition of feces classes of compounds analyzed

1. Proximates and minerals
2. Total, total soluble, and free soluble
3. Amino acid nitrogen
4. Creatine and creatinine
5. Total, total soluble, and free soluble individual amino acids
6. Tryptophan metabolites
7. Polyamines
8. Total and free fatty acids
9. Neutral steroids
10. Acid steroids

Results and Conclusions

It is difficult to adequately summarize all the data. An approach is to examine the statistically significant changes that occurred among the variables when the volunteers' diet changed from a meatless one to one containing a large quantity of meat. If, first of all, the two diets are compared, it is apparent that they differ significantly in several respects, (table 2). The table shows that in addition to protein, values for total calories, cholesterol and magnesium were greater in the high meat than the meatless diets. Carbohydrate and calcium values, on the other hand, were lower.

Table 3 provides a partial list of results obtained from analysis of feces of the volunteers. When one examines the variables listed in each of these categories, a pattern resulting from high meat consumption doesn't appear to emerge. It is interesting, however, that high meat consumption caused a significant increase in the magnesium content of the feces and a significant decrease in the calcium content. This corresponds with the higher magnesium content and lower calcium content in the high meat diet as compared with the meatless diet.

The high meat diet also contained more cholesterol than the meatless diet. Surprisingly, however, significantly less cholesterol and its derivative, cholestanol, were excreted by the volunteers during the high meat regimen that during the meatless regimen. The question then arises concerning the fate of the cholesterol ingested during the high meat diet.

Table 2. Food Composition

Values greater in high-meat than meatless diet	Values greater in meatless than high-meat diet
Calories	Carbohydrates
Protein	Calcium
Cholesterol	
Magnesium	

In examining the cholesterol content of the feces of the ten volunteers, it was observed that two of the volunteers excreted considerably more cholesterol than the eight other volunteers, irrespective of diet. The content of the degradation product of cholesterol, coprostanone, was slightly lower in the feces of these two volunteers than in the feces of the other volunteers. Thus, the ratio of cholesterol to degradation products was unusually high in two of the ten volunteers. Wilkins and Hackman (8) reported similar results in a study of the neutral steroid composition of the feces of 31 Americans on a normal diet. Approximately one-quarter of the subjects failed to convert cholesterol or plant steroids into degradation products

Table 3. Effect of Diet on Chemical Components in Feces

Values greater during high-meat than meatless diet	Values greater during meatless than high-meat diet
Magnesium	Calcium
Phosphorus	C 16:1 (total)
Creatine	Cholesterol
Spermine	Cholestanol
C 16:0 (free)	Coprostanone
C 18:0 (free and total)	Campesterol
C 18:2 (free)	Stigmasterol
Cystathionine (total soluble)	Cystine (total soluble)
Ornithine (total soluble)	O-Phosphoserine (free soluble)
Alanine (total soluble)	Threonine (free soluble)
Proline (total)	Glutamine (free soluble)
Glycine (total)	Serine (total)
	Glutamic acid (total)
	Methionine (total)
	Histadine (total)
	Arginine (total)
	I-Methylhistadine (total)

and were designated low converters. They reported that the feces of the high converters contained lower concentrations of cholesterol and higher concentrations of degradation products than the feces of low converters. We also observed this relationship.

Although analysis of the acid steroids is incomplete at this time, preliminary data indicate that diet does not appreciably affect the concentrations of these acids in the feces. Lithocholic and deoxycholic acids were present in the feces in relatively high concentrations under all dietary conditions and cholic and chenodeoxycholic acids were present in relatively low concentrations.

Dietary change did affect the composition of the microbial flora of the feces of volunteers, but not as we expected. All of the facultative and anaerobic bacteria isolated from the feces were identified to the species level. If one relates the total numbers of these two major categories of organisms, isolated from an individual, to diet, certain relationships are apparent. Figure 1 illustrates the influence of diet on the total numbers of facultative organisms isolated from each of the volunteers. It is clear that there were great variations in counts between individuals, but when all results were averaged, there were no significant differences in total counts from one diet to another. Somewhat different results were obtained when total anaerobic counts were analyzed. The influence of diet on the numbers of anaerobic organisms isolated from each of the volunteers is illustrated in figure 2.

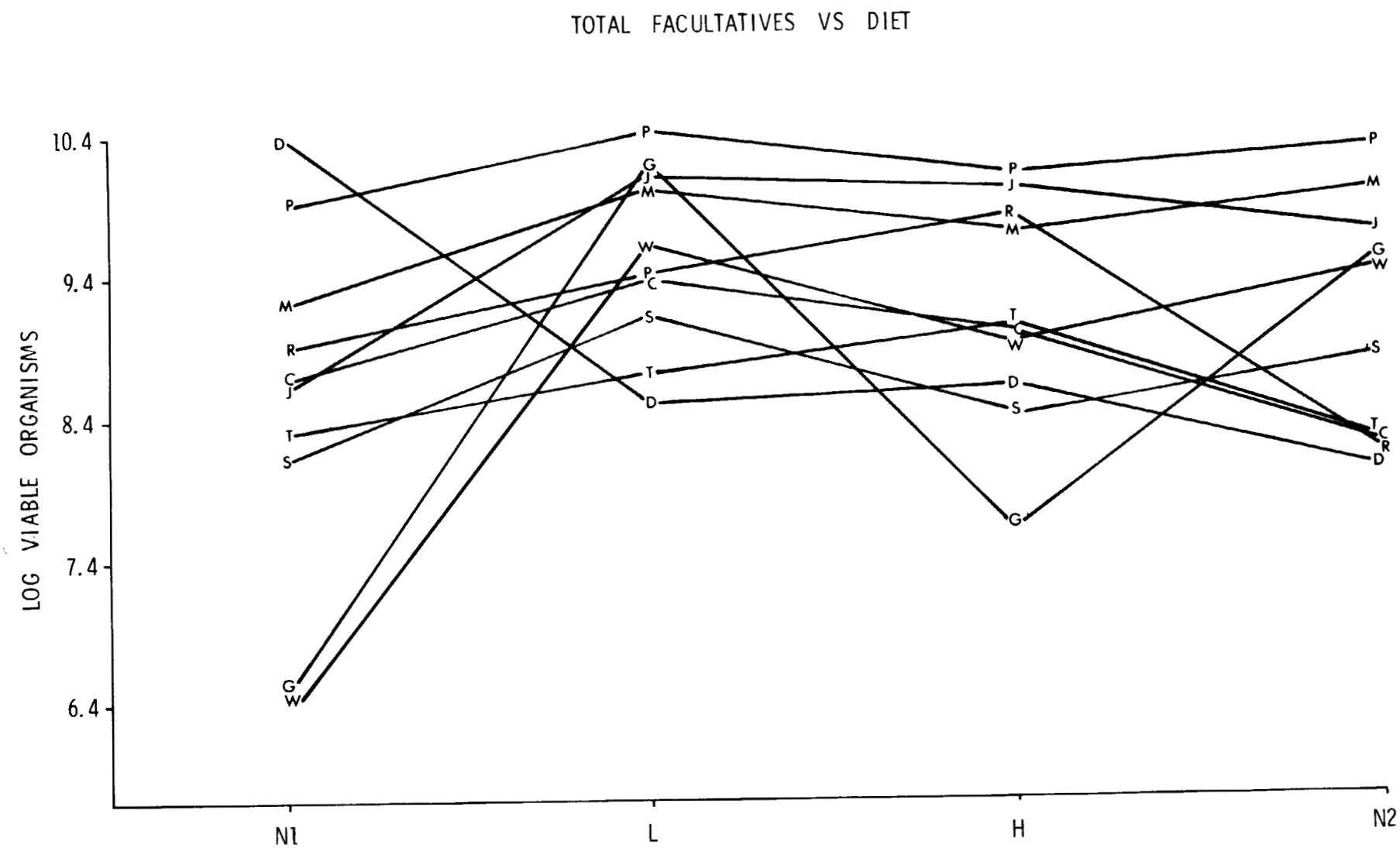

Figure 1. Influence of diet on the total numbers of facultative bacteria isolated from the feces of volunteers. N1 signifies normal diet 1; L, meatless diet; H, high meat diet; and N2, normal diet 2. Letters on the lines in the graph identify the volunteers.

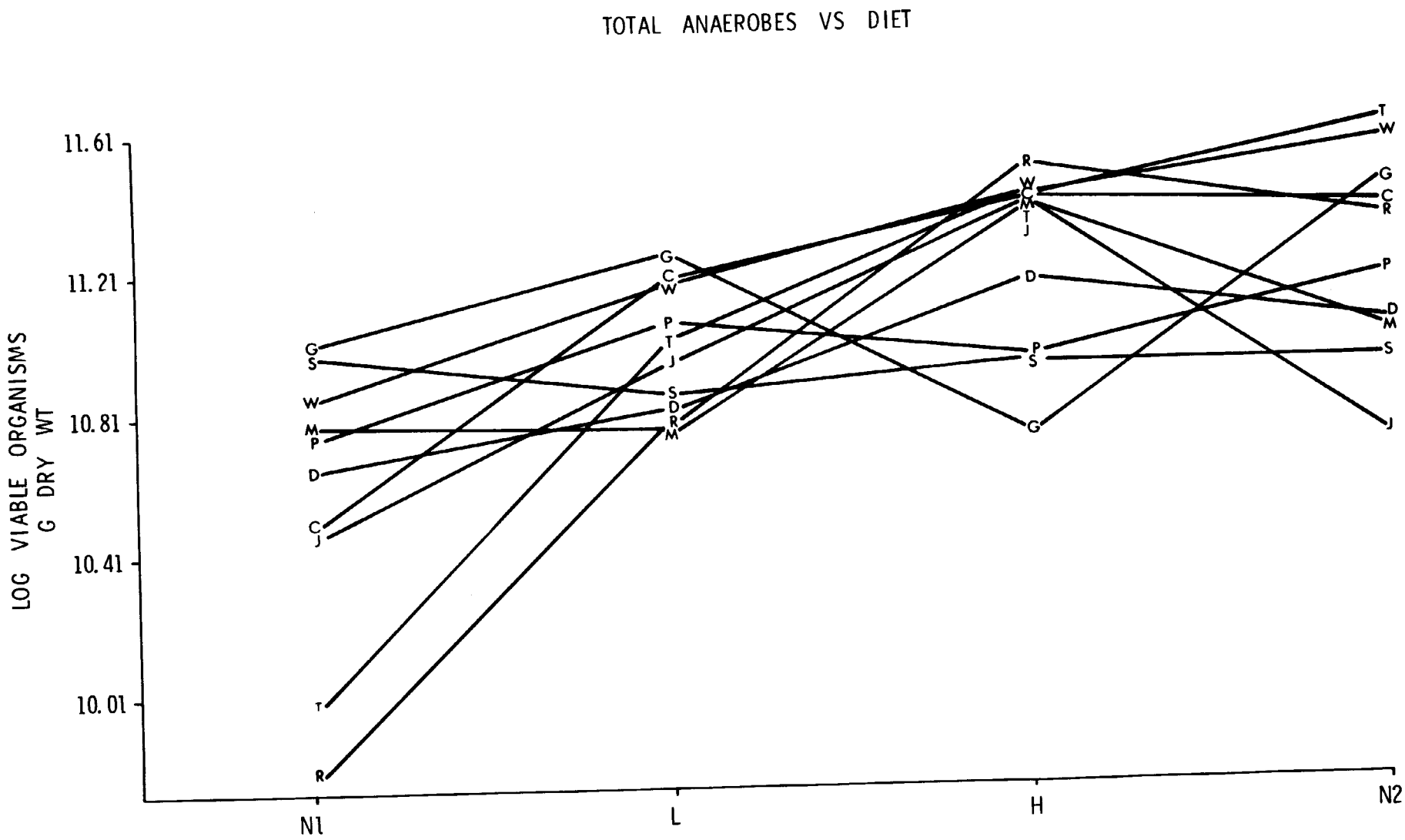

Figure 2. Influence of diet on the total numbers of anaerobic bacteria isolated from the feces of volunteers. N1 signifies normal diet; L, meatless diet; H, high meat diet; and N2, normal diet 2. Letters on the lines in the graph identify volunteers.

Again, we observe considerable variation in total counts from individual to individual. When results were averaged there was a gradual increase in total counts as the experiment continued. Thus, there was a statistically significant increase in total count as the individuals progressed from a normal diet to a meatless diet to a high meat diet and again to a normal diet. This relationship was observed twice when the diets were administered to different groups at different times. We have no explanation for it. We have speculated that it may simply represent a response to a regulated, institutional type of diet.

Analyses of bacteriological data are incomplete at this time. We are currently studying the effects of diet on the various genera and species of bacteria and may find that qualitative and quantitative changes occur among individual organisms when diet is altered.

What conclusions can we draw about the role of meat in colon cancer on the basis of the data at hand? Results we obtained were not anticipated in light of the reports of Hill and Aries (9) and Hill and others (6). We were unable, during the high meat diet, to demonstrate either an increase in the ratio of anaerobic to facultative bacteria or an increase in the concentrations of neutral and acid steroids, as we thought we might. According to the English group, all these factors have significantly higher values in the feces of British and Americans than in the feces of Indians, Japanese and Ugandans. It may be argued that our studies represent short term experiments and theirs, long term. However, Aries and others (10) reported that there are no differences in numbers and species of organisms in the feces of individuals consuming a mixed diet and in the feces of individuals on a strict vegetarian diet. The recent unpublished data of Moore at Virginia Polytechnic Institute and Finegold at U.C.L.A. also indicate that there is little difference in bacterial species in the intestinal tract of people of low risk and high risk populations. In addition to these observations, the degree of neutral steroid conversion in the intestine appears to differ among individuals even when they are on the same diet. (8) Thus, these long term experiments and our preliminary data fail to confirm the differences in flora composition and steroid concentration that the English group observed between high risk and low risk populations. However, in our studies, fat concentration was held constant in all four diets. The effects of high fat consumption on the acid steroid content of the feces needs to be determined.

Needless to say, the compounds that bacteria can produce in the intestinal tract are numerous. It is entirely possible that we failed to measure those, associated with a high meat diet, that are responsible for colon cancer. It is also possible that there is no relationship at all between meat consumption and cancer of the colon.

Summary

Ten volunteers completed a four month diet series consisting of one month each on a normal diet, a meatless diet, a high meat diet, and, again, a normal diet. During the fourth week on each diet, three stool specimens, collected from each volunteer, were analyzed for aerobic and anaerobic bacteria, proximates and minerals, amino acids, creatine and creatinine, tryptophan metabolites, polyamines, fatty acids, and neutral and acid steroids.

Preliminary data indicate that there was no increase in the ratio of anaerobic to aerobic bacteria or increase in the concentrations of neutral and acid steroids in the feces of volunteers during the high meat diet. Although this diet contained more cholesterol than the meatless diet, less cholesterol was excreted by the volunteers during the high meat consumption. Two of the volunteers excreted significantly greater quantities of cholesterol than the other eight, irrespective of diet.

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