on "G" during Pemmican feeding would arise by chance in less than 4 per cent of trials and those on "B" in less than 5 per cent of trials. The combined probabilities of the two experiments indicate that the higher values on fat feeding would arise by chance in something fewer than 2 out of 1,000 trials. The best estimate from these experiments is that the change to a high fat diet results in mean rises in serum cholesterol of 32.1 ± 10.8, and 31.3 ± 11.7 mg per 100 ml, for "G" and "B," respectively.

But Mann says that his use of the "t" test "revealed no evidence that subject B had influenced his serum lipid levels by this high fat intake. The cholesterol and S_f 12–20 levels of subject G indicated only suggestive evidence ($0.10 > P > 0.05$) that they had been increased by the treatment." How is this possible from these data? Perhaps in his computation Mann included the post-experiment observations as part of his "control," though he explicitly indicated he used *two* observations and not three.

But if the post-experiment observations are used we unwittingly introduce an additional hypothesis: "The post-experiment observations are not influenced by the previous high fat diet and are proper samples of the control universe." Can we prove this? On the contrary, computation indicates that these post-experiment observations are not homogeneous with the controls. Let us combine the observations on "G" and "B" by expressing the control and post-experiment observations on each subject as percentage of the mean control value for that subject. We have then, 4 control values, 101.0, 99.0, 105.5, 94.5, and 2 post-experiment values, 108.4, 108.0, the means of these being 100.0 and 108.2, respectively. But the standard errors of these means prove to be ±2.63 and ±0.16, standard error of the difference between means is ±3.42 and "t" = (108.2 − 100.0)/3.42 = 2.40. At 4 degrees of freedom this means that the odds are about 14 to one that the post-experiment values do not belong in the same universe as the control values.

—ANCEL KEYS, PH.D.
Laboratory of Physiological Hygiene
University of Minnesota

REFERENCES

1. MANN, G. V.: Lack of effect of a high fat intake on serum lipid levels. AM. J. CLIN. NUTRITION 3: 230, 1955.
2. CHANDLER, H. L., LAWRY, E. Y., POTEE, K. G., and MANN, G. V.: Spontaneous and induced variations in serum lipoproteins. *Circulation* 8: 723, 1953.

COMMENT BY DR. MANN

Dear Sir:

I believe that Keys is correct when he accuses me of bias in the interpretation of these data. These observations do indicate that the dietary treatment increased the serum cholesterol levels. It now appears to me that it would be more appropriate to compare the observed *differences* between control and treatment levels at the several observations rather than to compare the measured levels as I first did and as Keys so elegantly elaborated. The method with differences will show a probability of about 0.01 that these might be attributed to chance.

It is of interest to compare this limited experience—and these experiments were the extent of my facilities—with the variability we have observed in adult men in their usual environment both with and without dietary alterations. Since we have published these findings it is not necessary to use the plentiful but still undocumented estimates of Keys.

In the studies with Walker[1] we found that a short period of positive calorie balance accomplished with a very low fat diet increased the serum cholesterol levels of two young men 38 and 70 mg per 100 ml, respectively. In another experiment[2] in which three young men gained weight rapidly for 28 days while con-

suming large amounts of a high fat diet to which they were accustomed, the serum cholesterol level changes were +100, −20, and +50 mg per 100 ml. In a study of the diurnal variation of cholesterol in 17 adults done with Chandler,[3] the pooled standard deviation was 13.8 mg per 100 ml. In a study of normal subjects done with Watkin,[4] the mean total variation (S_T) over a period of several weeks of six young men of age and activities comparable to those of the present study was 14.64 mg per 100 ml (S. E. = 2.43). It is clear that the increases obtained in the "Pemmican" Studies were about twice the variation that has been found in comparable young men studied without treatment but that this effect of a high fat diet was considerably less than that usually produced by positive caloric balance.

The practical question, then, is whether the serum increases produced here are meaningful. In terms of the statistical hypothesis they seem to be, if we are willing to accept the several necessary assumptions. In terms of the variability of serum lipid levels over long periods and with diverse additional variables the increase observed here seems to me of questionable importance. I am skeptical that dietary fat is either the sole or even an important factor in the regulation of the serum lipid levels.

—GEORGE V. MANN, M.D.

REFERENCES

1. WALKER, W. J., LAWRY, E. Y., LOVE, D. E., MANN, G. V., LEVINE, S. A., and STARE, F. J.: Effect of weight reduction and caloric balance on serum lipoprotein and cholesterol levels. *Am. J. Med.* 14: 654, 1953.
2. MANN, G. V., TEEL, K., HAYES, O., MCNALLY, A., and BRUNO, D.: Exercise in the disposition of dietary calories: Regulation of serum lipoprotein and cholesterol levels in human subjects. *New England J. Med.* 253: 349, 1955.
3. CHANDLER, H. L., LAWRY, E. Y., POTEE, K. G., and MANN, G. V.: Spontaneous and induced variations in serum lipoproteins. *Circulation* 8: 723, 1953.
4. WATKIN, D. M., LAWRY, E. Y., MANN, G. V., and HALPERIN, M.: A study of serum beta lipoprotein and total cholesterol variability and its relation to age and serum level in adult human subjects. *J. Clin. Investigation* 33: 874, 1950.