lose. These extraneous carbohydrate substances are most often observed in the initial column effluent, particularly if water is passed through the column. Extraneous carbohydrates are also sometimes encountered in later effluents when liquids other than pure water are used. To reduce the amounts of these extraneous substances, it is suggested that cellulose columns be given a preliminary washing with water before use. While we presently know of no way to prevent completely the elution of extraneous substances from cellulose columns, we wish to call attention to their presence.

In operating chromatographic columns, particularly those of considerable length, it is desirable to apply positive pressure at the head rather than suction on the effluent. Application of vacuum often causes the lower portion of the column to be partially freed of solvent with consequent poor separations in these regions.

There seem to be two schools of thought concerning whether columns should be packed dry or by the slurry method. In our experience either method is good when properly handled. In packing by the slurry method care must be taken to use a thick slurry and thus prevent fractional separation of particle size or, in the case of a carbon-celite mixture, separations of the two components.

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References and Notes

1. R. L. Whistler and D. F. Durso, *J. Am. Chem. Soc.* **72**, 677 (1950).
2. L. Hough, J. K. N. Jones, and W. H. Wadman, *Nature* **162**, 448 (1948).
3. Celite No. 535, a product of Johns-Manville Co., New York, N.Y.
4. Such as Darco G-60, a product of Darco Corp., New York, N.Y.

14 October 1954.

Experimental Arteriosclerosis; Sulfur or Choline Deficiency?

A description of a kind of vascular abnormality produced in young rats by dietary means has been described by Wilgram, Hartroft, and Best (*1*). The authors attribute these arterial effects to a deficiency of choline, although the diets employed were profoundly deficient in essential organic sulfur compounds as well. Mortalities of 50 and 75 percent of the experimental groups in the 28-day periods of observation attest to the rigors of the diet. These experiments should be considered in relation to the chronicity of arteriosclerosis toward which all such experimentation is ultimately directed.

In their discussion the authors mistakenly observe, when referring to our work with experimental atherosclerosis in *Cebus* monkeys, that we have not specifically investigated the effects of choline deficiency on the vessels of monkeys. We have, in fact, done this and our observations were described (*2*). It was found that choline deficiency effectively *prevented* sufficient hypercholesterolemia to produce atherosclerosis in the monkeys. I believe that is because the animals became ill and refused to eat.

Our most atherogenic diets regularly contain 0.5 percent choline. We also observed (*2*) that this disease in monkeys is either prevented or cured with *l*-cystine and this effect has since been extended to several congeners of cystine with similar effects (*3*). It is perhaps of interest to those who press for the importance of choline in a variety of ailments that our discovery of the relationship of sulfur metabolism to atherosclerosis in monkeys resulted from the great difficulty we experienced in producing evidences of choline deficiency in this primate species.

Finally, Wilgram *et al.* propose that the much abused term *lipotropic* be extended to still another poorly understood phenomenon: the prevention of the accumulation of lipids in blood vessel walls. This redefinition of the term can scarcely do more than add to the existing confusion. The variety of meanings of *lipotropic* both in respect to anatomical structures involved and to the methods known or presumed to have induced the lipid deposition will require a short qualifying paragraph for each context in which the term is used.

It is well known that if a hungry cat consumes a saucer of cream, it will shortly show an accumulation of lipids both in its intestinal mucosa and in its liver. Are we to apply the term *lipotropic* to a milkman who does not come, to a more aggressive cat, or even to the large mouse that may have already satiated the cat? This proposal surely defeats the purposes of language.

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References

1. G. F. Wilgram, W. S. Hartroft, and C. H. Best, *Science* **119**, 842 (1954).
2. G. V. Mann *et al.*, *J. Exptl. Med.* **98**, 195 (1953).
3. G. V. Mann *et al.*, unpublished data.

5 August 1954.

We have had little experience with *Cebus* monkeys but are now starting an investigation of the role of various dietary factors in the production of atheromatous lesions in small primates. George V. Mann's previous studies have been of great help to us in planning this work. His present letter has raised several interesting points on which we would like to comment.

In the first place, Mann is discussing atherosclerotic lesions seen in monkeys that have been consuming diets high in cholesterol; we have described aortic medial sclerosis produced in rats by diets low in choline and essentially free from cholesterol.

He states that we attribute arterial lesions in choline-deficient rats ". . . to a deficiency of choline, although the diets employed were profoundly deficient

in essential organic sulfur compounds as well." Our hypolipotropic basal ration was designed to be as complete as possible with respect to essential amino acids while being *relatively* as low as possible in methionine. This diet contained 30 percent of alcohol-extracted peanut meal (about 50 percent protein), 10 percent of casein, and 5 percent of Alpha (soya) protein, giving 30 percent of protein; the total methionine of such a diet is about 520 mg, cystine is 340 mg, and amino-acid sulphur is just over 200 mg per 100 g of ration. Alpha protein at the 20-percent dietary level supplies about 200 mg methionine and less than 100 mg of sulfur. Despite the consumption by our rats of a diet containing more than twice as much methionine and organic sulfur as in the basal rations fed by Mann, they still developed aortic lesions.

We attributed the arterial effects in our experiments to choline deficiency because (i) the diets were known to be low in choline, and (ii) addition of choline to the basal diet consumed by control rats completely prevented development of lesions. The controls consumed amounts of the choline-supplemented diets comparable to the daily intake by the experimental animals of the basal choline-deficient diet. Controls did not receive any organic sulfur compounds other than those consumed by the experimental group. Therefore, in our experiments, the lesions in rats are clearly attributable to choline deficiency rather than to organic sulfur deficiency.

Mann says that our ". . . experiments should be considered in relation to the chronicity of arteriosclerosis toward which all such experimentation is ultimately directed" and draws attention to the rigors of our dietary regimen. The diets we employed were severely hypolipotropic, because they were designed to produce *acute* lesions *rapidly*. Less rigorous dietary regimens of the same type also produce in rats vascular lesions of the chronicity that Mann apparently desires. Experiments of this type were previously reported from our laboratories in 1952 [*Proc. Soc. Exptl. Biol. and Med.* **81**, 384 (1952)], incidentally antedating Mann's publications in this field [*J. Exptl. Med.* **98**, 195 (1933)]. The diets that we employed in the chronic type of experiment could be regarded as more "physiological" than those in the acute. Acute experiments are sometimes desirable, however, to speed up the progress of investigations of this type. The arterial lesions produced by both methods are identical, differing only in degree.

Mann states that they experienced "great difficulty . . . in producing evidences of choline deficiency in this primate species," and yet his paper contains several references to the fatty livers and poor condition of monkeys not given choline supplements. Surely his own data suggest that dietary choline (or its precursors including methionine) is necessary to maintain life of primates and that it is almost too easy to produce evidences of lack of adequate dietary choline in monkeys.

His own experiments elucidate to some degree the role of dietary choline in protecting the cardiovascular system of monkeys. We could find specific references in his paper to autopsy studies of only three monkeys (his numbers 1-6, 3-0, 3-2) that had been fed a hypolipotropic diet (low choline and low methionine, with 5 percent added cholesterol). Of these three animals two "showed minimal aortic lesions." These lesions had developed within 3 to 11 wk (average 7 wk) of dietary deficiency, despite the fact that the authors state "In the absence of choline animals had poor appetites, grew poorly and died with fatty livers." It is generally accepted that inadequate food intake will inhibit the development of lesions due to a deficiency of any vitamin or essential food factor. Therefore, in view of the relatively brief period of dietary deficiency and the poor food intake, we think it highly significant that under these conditions two or three monkeys developed even "minimal aortic lesions." Furthermore, seven control animals fed similar diets without added *dl*-methionine, but with 0.5 percent choline (see his Table VII) were free from aortic lesions. This series of 10 animals would indicate then that supplementary choline prevented lesions that otherwise developed in the experimental animals. His data with primates corresponds to ours obtained with rats in 1952.

Mann claims to have obtained a higher incidence of lesions in monkeys fed low-methionine, choline-supplemented diets containing 5 percent cholesterol. The total number of animals employed was greater but the *percentage*-incidence of lesions in this group was actually lower. These monkeys were observed for longer periods than were the choline-deficient group—for example, monkey No. 4-1 (from which *all* of Mann's photomicrographs were taken) was fed this diet for 25 wk. Of 17 monkeys in this group, eight developed aortic lesions demonstrable at autopsy; an incidence of less than 50 percent in these animals compared with 66 percent (two out of three) in the choline-deficient group. With such small numbers of animals, these differences may be of limited significance.

Mann found "that choline deficiency effectively *prevented* [his italics] sufficient hypercholesterolemia to produce atherosclerosis in the monkeys." But his own data show that in the absence of choline and *absence of hypercholesterolemia,* two of three animals did develop atherosclerosis. We reported [*Science* **119**, 842 (1954)] that choline-deficient rats fed diets *without* added cholesterol developed medial aortic lesions preventable by choline supplements. The choline-deficient rats had *lower* levels of serum cholesterol than the choline-supplemented controls. Therefore, under some conditions at least (two out of three of Mann's choline-deficient monkeys and in our experiments with rats referred to in foregoing paragraphs) aortic lesions developed without elevated levels of serum cholesterol. Therefore, we believe that the statement of Mann's quoted at the beginning of this paragraph needs some further support.

It should be noted that, to induce the atherosclerotic lesion in monkeys by use of diets high in cholesterol, a large intake of food appears necessary. To insure

adequate food intake it is essential to have choline in the diet, as Mann found. The aortic lesion we described, which does not require a sustained high food intake and which is prevented by choline, has a completely different pathogenesis.

Mann challenges our right and wisdom in broadening the meaning of the term *lipotropic*. We are most anxious to preserve the clarity of the term which one of us introduced. If choline deficiency leads to abnormal changes in liver, kidney, heart, and vessels that are preventable by adequate supplements with lipotropic agents (as the evidence published from this laboratory and from others now clearly indicates), we fail to see why the term should continue to be limited to the effects of deficiency on only one of these organs. Had the need for lipotropic substances in maintaining kidneys, hearts, and vessels been discovered at the same time as their need for maintaining the liver, Mann would probably not have objected to the broad concept we now propose for the term *lipotropic*. His objection is obviously based on the fact that these various manifestations of choline deficiency were discovered at different times. The proper designations for tardy milkmen and aggressive cats we prefer to leave to Mann's imagination.

Whether similar sclerotic lesions are under consideration and whether sulfur or choline is more important will require further careful experimentation. Interchange of data between responsible investigators will eventually provide the true answers, which may be quite different from Mann's present ideas or from ours.

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7 October 1954.

Potential Application of Community Censuses and Genealogies

I have been thinking that anthropologists whose records contain answers to two questions could help make possible some theoretical estimates of rates of ongoing evolution. These questions are: (i) How many people are there in the endogamous group? (ii) What proportion of individuals enter the group from without?

As I have pointed out ["Mixture and genetic drift in ongoing human evolution," *Am. Anthrop.* **54**, 433 (1952)], evolution has been defined as a change in gene frequencies. Gene frequencies change in response to a small number of factors: natural selection, mutation, admixture, and chance variation from one generation to the next. If ethnographers will provide answers to the two questions for a number of groups with a variety of cultures, one could calculate rates of admixture and chance variation. Under assumptions about mutation and natural selection, one could then estimate possible rates of evolution without any reference to the fossil record.

The minimum information necessary to estimate rates of accidental variations in gene frequency is a count of the total population of the endogamous group (be it community, tribe, or caste). In addition one would like to know, if possible, the proportion who are of reproductive age and status and the variation in number of offspring; that is, how many children survive to adulthood for each individual who completes procreation.

The minimum information necessary for a crude measure of admixture is the proportion of persons in the group who were born outside of it. A more useful form of the information is the tabulation of birthplaces of parents of all persons born in the group.

Many anthropologists have made a census or recorded genealogies. These have been preludes to other studies and are frequently unpublished or inadequately presented for the present purpose. However, some readers may be able to add another case. We do not even know yet whether human evolution would have been more rapid before cultivation of the land began. Perhaps we can at least find out to what extent, if any, hunters live in smaller more isolated reproducing groups than, for instance, agriculturalists.

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20 September 1954.

I know nothing more deadening to original ideas than keeping a man's nose firmly fixed to the grindstone. Even directors need a change, and young men should have opportunities of meeting other young men working in other parts of the country. Ideas are more likely to come from such meetings with colleagues than by holding men down to some work in which there might be no progress at all. No laboratory today is self-sufficing.—Lord Rutherford.