Table 3. Complement-fixation tests with virus and tumor antigens and antiserums. Virus antigens (V) were live viruses grown in tissue culture. Antiserums to virus were prepared by immunization of rabbits with live virus. Tumor antigens (T) were saline extracts of virus-induced tumors, and serums were obtained from tumor-bearing hamsters. Results are expressed as reciprocals of the highest dilution of antiserum reaction with antigen.

Antigens	Serums							
	SV20V	SV20T	SV38T	SA7V	SA7T	SV40T	SV40T†	Normal
SV20V	64	<2	*	*	*	*	<2	<2
SV20T	<2	32	4	*	<2	<2	<2	<2
SV38T	*	4	32	*	*	*	*	*
SA7V	*	*	*	32	<2	*	*	*
SA7T	*	<2	*	<2	32	4	*	<2
SV40T	*	<2	*	*	<2	256	*	<2
SV40T‡	<2	<2	*	*	<2	*	*	<2

* Not tested. † This serum was supplied by Dr. Bernice Eddy, NIH. ‡ This antigen was supplied by Dr. David Axelrod, NIH.

no naturally occurring primates other than man. A lower incidence (19 percent) was reported in a U.S.A. population. These findings, combined with the demonstration of a potent viral oncogen (SA7) occurring in primates in Africa, suggest that epidemiological studies of the relationship of SV20 and SA7 to lymphomas of the Burkitt type, and to other malignancies in these divergent geographical areas, would be of considerable interest.

Earlier reports on the oncogenicity of SV40 for baby hamsters had a profound effect on the production and testing of virus vaccines prepared in cell cultures from monkey kidney, as judged by the more stringent regulations that have been imposed upon manufacturers of such products. Although the simian adenoviruses do not appear as contaminants in primary cultures of monkey-kidney as frequently as does SV40, our findings point to possible contamination of products for human immunization with at least seven different viruses of demonstrated oncogenic potential. Thus, the program recommended by the Committee on Tissue Culture Viruses and Vaccines (appointed by the director of the National Institutes of Health) for the valuation of serially propagated cell cultures for vaccine preparation (*11*) should be vigorously pursued.

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Bacterial Contamination of Some Carbonaceous Meteorites

Abstract. *Three types of bacteria were isolated from samples of two carbonaceous chondrites and identified as common contaminants that are widely distributed.*

As an extension of other work done in our laboratory on carbonaceous chondrites (*1*), it was considered of interest to determine to what extent, if any, these meteorites are contaminated by ordinary viable microorganisms. The following three meteorites were selected for investigation. The Orgueil (Wiik type I) received from A. Cavaillé, the Murray (Wiik type II) obtained from E. P. Henderson, and the Mokoia (Wiik type III) secured from C. B. Moore. Using standard microbiological techniques, we had no difficulty in obtaining bacterial cultures from samples of the Murray and Mokoia but were unable to detect the formation of any bacterial colonies from samples of the Orgueil.

Small fragments of each meteorite (*2*) were removed aseptically and placed in carefully weighed sterile containers. One milliliter of sterile water was added, and the tubes were placed on a rotary shaker for 30 minutes. The supernatant was carefully removed and plated on nutrient agar. The plates were incubated 24 to 48 hours at 37°C. The colonies were counted on a Quebec counter. The meteorite fragments were then dried, reweighed, and ground to a powder with a mortar and pestle that had been washed in acid or autoclaved. (In other tests the fragments were ground without any prior treatment). One milliliter of sterile water was again added, and the sample was treated as before.

The results are summarized in Table 1. Microbiological analyses of the Murray samples gave consistent results, whereas analyses of the different Mokoia samples were more variable; no viable growth could be found in any of the Orgueil samples tested under either aerobic or anaerobic conditions. Some Orgueil samples were incubated at room temperature for 2 weeks with no additional change. We cannot say, however, whether more prolonged incubation might have shown growth of fungi or other microorganisms that require unusually long incubation periods for germination.

As shown in Table 1, the approximate average contamination of the samples ranges from 1800 (Mokoia) to 6000 (Murray) bacteria per gram of meteorite. The organisms isolated from the Murray were identified as *Bacillus cereus* and *B. badius* (in a ratio of 5 to 1) and the organism from the Mokoia as *Staphylococcus epidermidis*. Identification was accomplished by extensive testing according to the descriptions given in Bergey's manual. No organisms were isolated from the solvent or the agar.

It appeared that a much higher bacterial count, in comparison with the count from the supernatant fractions, could be obtained by spreading the washed meteorite granules on nutrient agar; hence affinity between the meteorite particles and the bacteria is suggested. This observation is in line with reports by other investigators of bacteria and other microorganisms that grow out from hard rocks and minerals into nutrient media (*3*). The spreading of the meteorite particles on agar plates was not employed for determining the bacterial counts because of the additional work and the difficulty

of differentiating between the meteorite particles and the bacterial colonies. It should be noted that since the transfer of the bacteria from the meteorite solid matter to the water was not quantitative, the numbers given should be taken only as lower limits of contamination.

The isolated bacterial samples were fixed on standard microbiological slides. The slides were stained by standard Gram-staining procedure and studied at 930 magnification. Figure 1 shows the two species of bacilli found in Murray, and the single species of cocci found in Mokoia. These slides were compared with slides of the ground meteorite samples, which were similar to those already described (*4*). We think it would be unwarranted on the basis of morphology alone to draw a relationship between the two types of slides even though there appeared to be a certain similarity in size and general appearance of several particles. It may be of interest to point out, however, that the extent of contamination by viable bacteria observed in the Mokoia and Murray meteorites was approximately 3 percent to 25 percent of the number of presumably nonviable bacteria ("apparent-contaminants") observed by other investigators in other meteorites (*5*).

The tests were repeated 2 months later, and exactly the same results were obtained—that is, Orgueil was negative; Mokoia gave few colonies of a single organism; Murray gave a large number of colonies of the two bacilli. The consistent negative results obtained with the Orgueil samples as well as with the controls definitely show that no contamination took place while the microbiological testing was being carried out. Furthermore, the reproducibility of the results after a period of 2 months indicates that the organisms have been associated with the meteorites for some time and that no significant contamination is taking place in our laboratory.

It should be noted that the Murray meteorite, which, according to E. P. Henderson (*6*) has had a high probability of contamination, is the one which appears most contaminated. The results from the Mokoia meteorite, showing less growth and greater fluctuation from sample to sample, suggest a lesser degree of contamination; this meteorite has been subject to a minimum amount of handling and exposure to the atmosphere. The absence of viable organisms in the Orgueil sample may be a reflection of the fact that the sample was well preserved in a sealed container in the Mountaban Museum and has had little chance of recent contamination in our laboratory. The microbial contamination of the Murray and the Mokoia meteorites could have occurred recently or, more probably, some time ago. In addition to the evidence described above in favor of the latter, it should be said that there is no problem in assuming that it occurred months or even years ago since it is well known that the spores and vegetative forms of many species of bacteria may survive for several years under ordinary conditions; survival for 37 years has been reported for spores of bacillus species (*7*). It is also known that bacteria can penetrate hard rocks (*3*, *8*) and meteorites (*8*).

Even though the presence of viable organisms in meteorites has been claimed from time to time, with an implication that the organisms were indigenous to the meteorite (*9*), no identification of these organisms has been

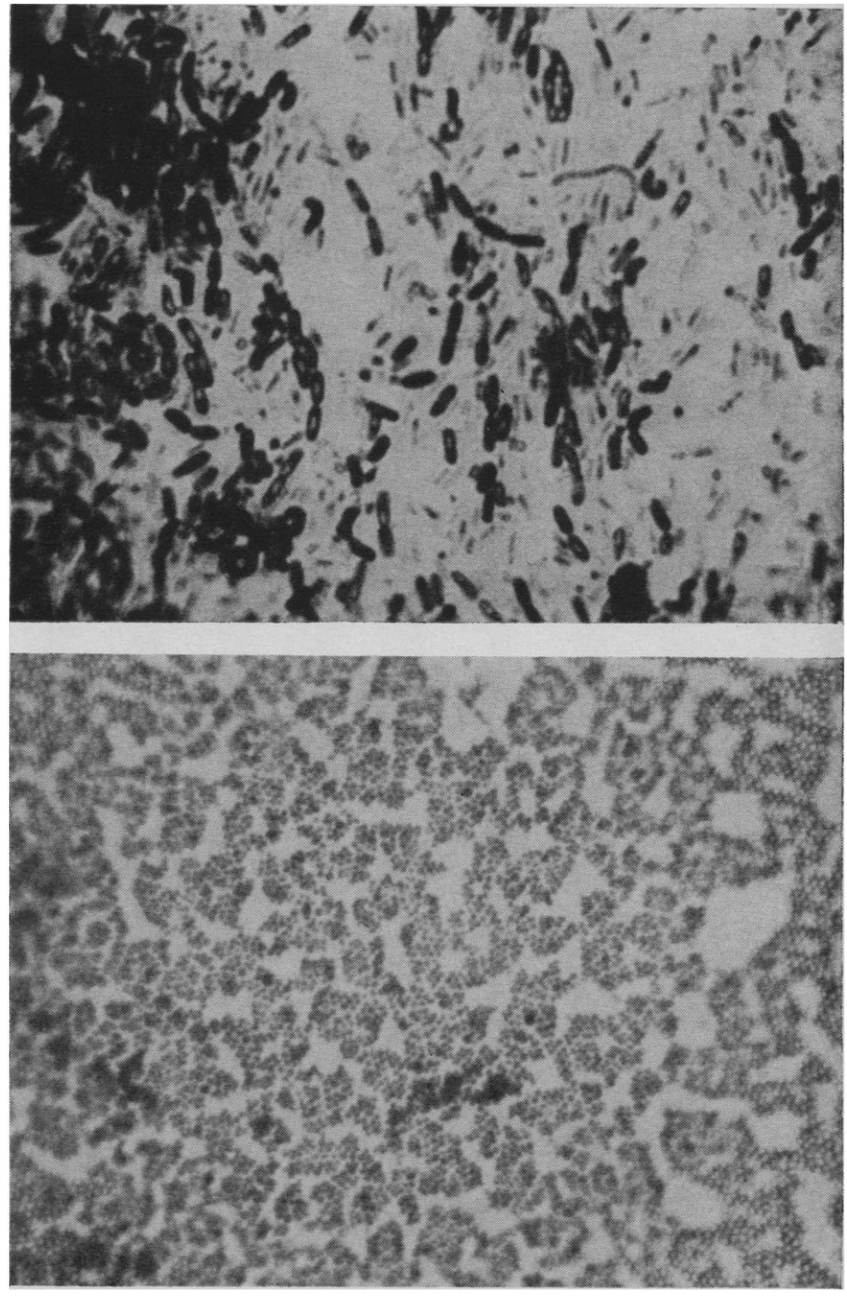

Fig. 1. Slides of 4-day-old cultures of bacterial contaminants (× 930). (Top) *Bacillus cereus* and *B. badius* isolated from the Murray sample. (Bottom) *Staphylococcus epidermidis* isolated from the Mokoia sample.

Table 1. Bacterial cultures isolated from carbonaceous meteorites. Each value represents the average of four different determinations on four different small samples of the same meteorite. No colonies were obtained from the solvent or the nutrient agar.

Sample	Wt. (10^{-3} g)	Colonies per milliliter	
		Fragment washings	Powder washings
Murray	89.5	0	530
Mokoia	84.6	20	150
Orgueil	138.7	0	0

published in the scientific literature except that of Roy (*10*), who found *Bacillus subtilis* and *Staphylococcus albus* as contaminants in the Johnstown meteorite, a noncarbonaceous, achondritic stone. Our report constitutes the first definite identification, known to us, of viable ordinary bacterial contaminants in carbonaceous chondrites, specifically the Murray and Mokoia meteorites. It is of interest that similar strains of microorganisms have been found in these two independent studies, despite the fact that the meteorites examined are quite different.

Although the number of viable contaminants per gram of meteorite is relatively low, it remains to be seen whether the intermittent and repeated contamination of carbonaceous chondrites by microorganisms, which eventually leave their residues in these stones, may have contributed to any significant degree to the formation of some of the organic compounds which have been detected in these meteorites (*4*). On the basis of this and related work (*7–10*) it appears that future analyses of organic compounds in meteorites should be preceded by careful microbiological examinations.

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Exchangeable Mass: Determination without Assumption of Isotopic Equilibrium

Abstract. *The method of isotope dilution for determining the exchangeable mass of different substances in living organisms is, at best, approximate. A formally exact method, defining the otherwise vague concept of exchangeable mass, is proposed. The new method may serve as a standard for determining errors in results obtained by the earlier method.*

In considering potassium metabolism in man, if we assume steady state, the total potassium pool (that is, the body's total content of potassium) is constant in time. This pool may be regarded as two mutually exclusive subpools: exchangeable and nonexchangeable potassium. Total body potassium can be determined, at least in principle, by whole-body counting techniques, and usually the exchangeable mass has been determined by so-called isotope dilution. However, as explained below, the dilution method is based on a theoretically incorrect assumption, and in this report I present an alternative method in which this assumption is relaxed. The theory precisely defines exchangeable mass and gives some rules for design of experiments; for instance, the form of tracer supply may be significant (a special concept, "equivalent tracer supply," is introduced for this purpose).

In the following discussion, the population of isotopes naturally present in the body is referred to as "mother substance." The dilution method proceeds as follows: A small amount of a ^{42}K salt is injected into the body; the population of injected ions ($^{42}K^+$) is referred to as "tracer." The total-body content of tracer is observed, as well as the specific activity of blood (specific activity is the ratio between the amount of tracer and the corresponding amount of mother substance). It is assumed that after a time—perhaps 24 hours—"isotopic equilibrium" is reached; that is, specific activity is equal throughout the exchangeable potassium pool. On the basis of this assumption, measurements made at that time will permit determination of the amount of mother substance in the exchangeable pool; the intuitive concept of exchangeable mass thus becomes operationally defined.

In 1956 Berman and Schoenfeld (*1*) showed that the idea of isotopic equilibrium in terms of equal specific activity is justified only when the system is closed (an additional necesary assumption is that the system is irreducible). The metabolic system considered here is open, and it is then necessary to accept the dilution method as an approximation: one assumes that the specific activity is *approximately* the same throughout the exchangeable potassuim pool. There is no obvious reason to believe in such an approximation (*2*) and—this point is crucial—there has been no way to estimate the degree of approximation (*3*). To test the dilution method by killing animals and analyzing the ash is wrong in principle because the analysis gives total rather than exchangeable potassium. The occasional close agreement between results obtained by the dilution method and by ash analysis merely suggests that the former produces overestimates, in agreement with what theory indicates (*4*); this view is supported by work on sodium and potassium in man (*5, 6*).

Although specific activity in an open system everywhere approaches zero with time, and in that respect ultimately becomes everywhere equal, this fact does not justify the dilution method. The method requires both that the difference between specific activities of two arbitrary parts of the system should be zero, and that the ratio between the two specific activities should equal unity. This is more precisely explained later: it suffices here to note that it is