macerated sheep brain, successfully transmitted the disease in serial passages through *Macacus rhesus* monkeys by means of cultures of the virus in dilutions ranging from 2×10^{-7} to 2×10^{-27} of the original material.

In view of what has been discussed it is now apparent that the asserted lifeless culture medium of Eberson can not be regarded as such. The observations and conclusions drawn from that study are explainable only on the basis of a modified tissue culture which was capable of supporting the existence of poliomyelitis virus and enabling it to multiply in the presence of viable and susceptible cells. Hence the results which embodied transmissibility in series, infectivity and immunological considerations were not surprising or unexpected. A reexamination of the original protocols and a scrutiny of the photomicrographs which illustrated the article indicate clearly that the visible bodies or "organisms" multiplied in and about the tissue particles, the nuclear elements especially. This was stated unequivocally in the text with regard not only to the subculturing of the material in serial dilutions, but also with reference to the type of inoculum employed in the transmission of the disease to monkeys. An experiment performed in 1931 at the Rockefeller Institute,[8] with a submitted culture in the eighth subplant representing a dilution of the original inoculum of approximately 2×10^{-17}, emphasized this point. There it was decided to inject intracerebrally in a monkey one cubic centimeter of the supernatant fluid from lightly centrifuged *culture material which had previously been ground in a mortar with sterile quartz sand.* The infective power of such a culture was successfully demonstrated when poliomyelitis developed after one week in the test animal, from which in turn it was possible to transmit the infection to another monkey with material derived from a suspension of brain and nervous tissue. The intimate relation of the multiplying virus to the tissue particles of the culture medium was thus indubitably shown.

With regard to the brain tissue medium itself it was stated that it must have been lifeless in consequence of the mode of sterilization. This assumption, despite subsequent thermal controls, was erroneous. Adequate heat penetration was made difficult, owing to the nature of the medium and its containers during the process of sterilization. That there was considerable variation in the different lots of medium as a result of this would follow from the irregular successes of some infectivity experiments, particularly in the later subplants from cultures beyond the tenth generation. Supplementary experiments designed to cultivate the virus with thoroughly cooked culture medium prepared in another laboratory resulted in failure. This was to be expected from the nature of the virus and thus confirmed the fact that the substrate used originally could not have been lifeless in the accepted sense.

Regardless of present or future attempts to cultivate certain viruses in lifeless media, and assuming this as only remotely possible, much knowledge concerning their behavior and properties can be gained nevertheless by a study of viruses in a tissue culture medium or some modification of it. This has been amply demonstrated recently for the infective agents of vesicular stomatitis,[9] poliomyelitis,[10] psittacosis[11] and louping ill,[12] all these having yielded to cultivation in media similar to that used for vaccine virus.

In discussing this subject it is recognized that the difficulty in cultivating a virus is related directly to its degree of parasitism. Subject to this condition it should be possible to devise methods of study with culture media adapted to the needs of individual viruses. Considering their widely divergent behavior in the animal and human tissues, is it not paradoxical to suppose that all viruses in common should conform to a single type of cell-host parasitism?

A study is now in progress to determine whether or not the virus of poliomyelitis can be adapted to cultivation in various modifications of culture media containing suitable tissue and physiological fluids. It should be of some interest to ascertain the possible relationship between the ability of a given virus to multiply and its reputed degree of parasitism, to the end that a "parasitic index" for viruses in general might be evaluated.

FREDERICK EBERSON

101 GROVE ST.,
SAN FRANCISCO, CALIF.

[8] F. Eberson, *Jour. Lab. and Clin. Med.*, 18: 586, 1933.

[9] H. R. Cox, J. T. Syverton and P. K. Olitsky, *Proc. Soc. Exp. Biol. and Med.*, 30: 896, 1933.

[10] E. Gildemeister, *Cent. Bakt.*, Abt., 1, Ref., 109: 284, 1933.

[11] S. P. Bedson and J. O. W. Bland, *Brit. Jour. Exp. Path.*, 15: 246, 1934; J. O. W. Bland and R. G. Canti, *Jour. Path. and Bact.*, 40: 231, 1935.

[12] T. M. Rivers and S. M. Ward, *Proc. Soc. Exp. Biol. and Med.*, 30: 1300, 1933.

THE VITAMIN C CONTENT OF APPLES AND ITS RELATION TO HUMAN WELFARE

IN view of the extreme variety differences in vitamin C content of apples[1,2,3,4] it has been pointed out[5] that the exchange of certain varieties of apples

[1] M. F. Bracewell, E. Hoyle and S. S. Zilva, *Biochem. Jour.*, 24: 82–90, 1930.

[2] M. F. Bracewell, E. Hoyle and S. S. Zilva, *British Med. Res. Council, Sp. Rpt. Ser. B.*, 146: 3–145, 1930.

[3] C. R. Fellers, P. D. Isham and G. G. Smith, *Proc. Am. Soc. Hort. Sci.*, 29: 93–97, 1932.

[4] G. G. Smith and C. R. Fellers, *Proc. Am. Soc. Hort. Sci.*, 31: 89–95, 1934.

[5] W. Franklin Dove, *Am. Nat.*, 69: 469–544, p. 524, 1935.

for other varieties would deprive 200,000 to 440,000 people (depending upon production rates) in one particular apple section (Maine) of their yearly vitamin C supply.

This fact becomes increasingly important because of attempts which have been made in recent years to bring about cooperative production (and distribution) whereby apple growers in delimited areas of the country have been encouraged to combine in their efforts and concentrate upon the production of single varieties. That the correct variety of fruit be selected for cooperative production is manifestly important to the welfare of the consuming public. In some regions the choice of varieties unfortunately has been made in favor of a low-vitamin apple.[6] There is a trend toward low vitamin C apples in various parts of the country. At the present time there are more trees of the Delicious variety than of any other variety in the United States.[7] The Delicious is one of the varieties poorest in vitamin C.

The cooperative-production plan may be economically sound, but if put forth without due regard to vitamin selection will certainly prove to be biologically unsound—especially in regions where citrus fruits are not grown and are therefore expensive, and since food habits and cooking methods can only slowly be readjusted to harmonize with the most recent information secured from vitamin research and health surveys. Other nutritional factors being equal, varieties of fruits and vegetables adapted to the particular geographical environment and high in vitamin content would be desirable.[8] In regions adapted to apple production, the apple could be depended upon as the principal source of antiscorbutic vitamin, a source of special value because the apple is generally consumed raw.

However, attempts to take advantage of vitamin research in the apple industry bring out some difficulties which tend to discourage the consideration of vitamins in fruit-breeding programs. Some agriculturists feel that the apple breeder must follow the preference of the consuming public and that if the public desires a certain variety, that variety it must have, regardless of low vitamin content. It might, of course, be pointed out that the results of extensive experiments[5] demonstrate the fact that proximity can divert the nutritive desires of the individual. But an answer more likely to be accepted by those loath to educate the consumer is that vitamin potency should be bred into varieties that are already acceptable to the consuming public.

[6] *Maine Ext. Bul.*, 214: 1–19, 1934.

[7] C. C. Thompson, *Wash. State Agr. Exp. Sta. Bul.*, 277: 1–108, 1933.

[8] W. Franklin Dove, *Maine Agr. Exp. Sta. Bul.*, 375: 191–284, p. 268, 1934.

However, at this point a second difficulty arises. It is claimed that the generation process in the fruit tree takes too long a time to permit of positive vitamin work. It is true of course that the time will be no longer, perhaps, than the time required to alter the food demands of the population. Still, however, the difficulty is a real one. Could an experimental method be found whereby the generation interval might be reduced from the present requirement of 12 to 15 years to one of a year or less, many advantages would ensue.

It has occurred to the writers that should the characteristic vitamin C content of the fruit be a characteristic also of the sap, leaf, stem or root tissues or of other non-fruit parts of the tree, a part of the seedling could be tested as an indicator of the future vitamin potentiality of the fruit. The gain in time might make vitamin research a feasibility in apple-breeding plans.

In the present communication the results of preliminary tests made on the ascorbic acid content of leaves of two varieties of apples—one high in vitamin C (Northern Spy) and one low in vitamin C (McIntosh) are here presented. The titrimetric method, using the dye indicator, 2, 6-dichlorophenol indophenol, in the technique as modified by Bessey and King,[9] was followed with a change from the hot acetic acid to cold acetic acid for stabilization of the vitamin. The determinations of the ascorbic acid content of the leaves were made in September and October during and after apple harvest time.[10] On 17 different days the leaves were tested from these two varieties, the Northern Spy and the McIntosh. In 14 of the 17 tests the ascorbic acid content of the Spy leaves exceeded that of the McIntosh leaves. The mean difference in ascorbic acid content between the two varieties was $0.246 \pm .0682$ mg. of vitamin C per gm of fresh leaves. The odds were 68.4:1 that the differences were significant. The results of 15 tests made after the adaptation of the technique to the material gave an average ascorbic acid content of .7457 mg per gm for the McIntosh and 1.1203 mg per gm for the Northern Spy. Thus the vitamin C content of the leaves bears a direct relation to the vitamin C content of the fruit. The Northern Spy apple is from five to six times as potent in vitamin C as is the McIntosh apple (4 to 6.5 grams of Northern Spy apple equals 20 to 25 grams of McIntosh apple[3,4,11]).

[9] O. A. Bessey and C. G. King, *Jour. Biol. Chem.*, 103: 687–698, 1933.

[10] The writers are indebted to both Mr. R. M. Bailey, of the Experiment Station, and Professor J. H. Waring, of the horticultural department of the University of Maine, for plant materials used in these tests.

[11] E. L. Batchelder, *Jour. Nutr.*, 7: 647–655, 1934.

Further tests are in progress in order to determine the earliest age at which these differences in ascorbic acid content of the leaves or other non-fruit parts of the plant are detectable. If the method proves successful and adaptable to other fruits and vegetables, it will make possible a "vitamin sieve" to precede all other tests of adaptability, winter hardiness, consumer preference and trade demands.

W. FRANKLIN DOVE
ELIZABETH MURPHY

BIOLOGICAL LABORATORY
MAINE AGRICULTURAL EXPERIMENT STATION
UNIVERSITY OF MAINE, ORONO

DETERMINATION OF THE CHLOROPLAST PIGMENTS OF PLANTS

THERE is considerable evidence at hand on the possible relationship of the chloroplast pigments, especially the carotinoids, to sexual reproduction in plants.[1] To minimize discouragement in this type of study[2] and the drawing of premature conclusions one should exercise particular care in methods of determination of the carotinoids, a significant percentage of which may be lost in the process of extraction and purification. Of even greater importance is the selection of proper material for the quantitative analysis of these pigments. Carotene, for instance, seems to increase in concentration (Murneek—soybean leaves) and in quantity (Virtanen *et al.*—peas and wheat) till the time of flowering and early fruit setting and then decreases rapidly. Hence the mere determination of these pigments in "fruiting" and "vegetative" plants loses significance, unless their developmental states are carefully correlated with the analytical assay. In fact, at certain stages of growth the fruiting plants may have less carotene than the non-fruiting ones.

A. E. MURNEEK

COLLEGE OF AGRICULTURE,
UNIVERSITY OF MISSOURI

VERTEBRATE LOCALITIES IN SOUTH PARK, COLORADO

DURING the past three summers we have been cooperatively engaged in a geological study of South Park, Colorado. This project was financed at first by grants from Northwestern University and from anonymous contributors, and brought to a successful conclusion, thanks to a grant from the Geological Society of America. With the permission of the society, the following brief account is published as of interest to stratigraphers and especially to vertebrate paleontologists.

The basin of South Park has been the site of subaerial and fresh-water deposition at various times since the Laramide Revolution. There are four localities where we have found fragmentary but identifiable vertebrate remains. Since, for lack of time, only short periods (a day or less in any one place) were devoted to search, there is reason to believe that any of these localities may yield better remains when more carefully examined.

Three of these localities appear to represent beds of White River age and are in what we have tentatively designated the Antero beds. In the southwest quarter, section 33, T. 12 S., R. 76 W., tuffaceous beds above a conglomerate bore fragments of a tooth identified by Mr. C. W. Gilmore, of the National Museum, as that of a titanothere or possibly uintathere of Oligocene or late Eocene age. In the southwest quarter, section 8, T. 14 S., R. 75 W., is a cut exposing fine tuff and gritty, blocky clays, mostly white to light gray in color, which aggregate about 120 feet in thickness; from these beds teeth, skull bones and limb bones were collected, representing six mammalian forms and pronounced by Mr. C. L. Gazin, of the National Museum, to be of White River (Oligocene) age. About a mile east of the Fairplay-Antero Junction highway in section 22, T. 10 S., R. 77 W., on the east side of a flat-topped ridge, white tuff beds are capped by pinkish sandy clays with local conglomeratic layers. From the pink beds several small fragmentary jaws and teeth were collected, representing chiefly insectivores, marsupials and artiodactyls, and referred by Mr. Gazin to White River age.

Immediately south of the park, on the divide separating South Park from Wagon Tongue Creek, in sections 31 and 32, T. 14 S., R. 75 W., gravelly and clayey beds, having a thickness of about 250 feet and tentatively called by us the Wagontongue beds, are well exposed in two northward-facing cuts. From the eastern exposure one jaw representing an Equid was collected and on this basis Mr. Gazin referred the beds to the upper Miocene or Pliocene.

The collections from these localities have so far been given tentative study only, but faunal lists are available. Our project does not include further collecting in the near future. We publish this note in the hope that others may find it possible to devote more time to search for fossils in the places listed. All except the last are readily accessible by secondary roads. If

[1] S. Satina and A. F. Blakeslee, *Proc. Nat. Acad. Sci.*, 12: 191–196 and 197–202, 1926. R. Chodat and W. H. Schopfer, *Comptes Rendus Soc. Phys. et Hist. Nat.*, 44: 176–179, 1927. M. Cajlahjan, *Comptes Rendus Acad. Sci. U. S. S. R.*, 1: 1:40–42, 1932. A. T. Virtanen *et al.*, *Biochem. Zeitschr.*, 267– 1–3: 179–191, 1933. A. E. Murneek, SCIENCE, 79: 528, 1934.

[2] SCIENCE, 82: 596, 1935.