intervals by microscopic observation of stained cells grown on cover glasses in Leighton tubes, and by direct counts, for cell increase.

In some of the tests $NaHCO_3$ (0.0002 gm/ml) was added and filtered as a component of the medium. In other tests the bicarbonate was filtered separately and then added aseptically to the medium to give a final concentration of 0.002 gm/ml. This difference in bicarbonate concentration produced such a striking difference in growth response that the former was used as a minimal medium while the latter was used as an optimal medium.

The GP factor was active over a concentration range of 10 to 250 μg/ml; however, the optimal concentration was 100 μg/ml. Toxicity was encountered when the concentration exceeded 250 μg/ml.

In the minimal medium containing 10 percent horse serum, the time required for the cells to attach and flatten to the glass was about 48 hours and the generation time was approximately 40 hours unless the inoculum exceeded 20,000 cells/ml. As the serum concentration was reduced, the flattening and generation times were increased, until at 2.5 percent the medium was merely a maintenance medium in which no cell increase was observed. Addition of the GP factor to the medium with 2.5 percent serum produced an increase of one generation at 8 days. When the GP factor was tested with the serum concentration at 5.0 percent, the growth was equal to that obtained with cultures grown in medium containing 10 percent serum, and exceeded that of the 5.0 percent serum control by 1½ generations at 8 days. It was evident that the GP factor was supplementing or partially substituting for some component of the horse serum.

When these cultures were examined microscopically by using stained preparations, the 4-day cultures revealed that 8.7 percent of the cells were in some stage of mitosis. Those grown in 10 percent serum and in the absence of the factor had only 0.3 percent of the cells in mitosis. At 6 days the difference in the number of mitotic cells had decreased to 2 percent, and at 8 days there were more cells in mitosis, on a percentage basis, in the cultures without the factor.

When GP factor was added to the optimal medium (tenfold increase in $NaHCO_3$) with 10 percent horse serum there was no significant improvement in flattening or growth time. The GP factor growth response was, however, apparent when the serum concentration was reduced to 7.5 percent or lower. No difference in flattening time was seen at any serum concentration tested.

While the GP factor is not a chemically defined compound, it does represent a material of potential biological importance, and any knowledge of its structure could be of value. The material is remarkably difficult to analyze by routine chemical tests. The only evidence for chemical structure was obtained from bacterial assays where the material was subjected to tests for stability to certain chemical treatments. Loss of activity as a result of treatment was presumed to indicate reaction with the test agent. Suitable toxicity controls containing GP factor were included. The results of these tests suggest that the active substance is a carboxylic acid possessing at least one hydroxyl group and certain unsaturated bonds which are requisite to activity. Evidence from growth tests for or against a carbonyl group was inconclusive, although tests with $AgNO_3$ and Schiff's reagent were weak or negative, as was the $FeCl_3$ test for phenolic or enolic compounds. Growth tests using mammalian cells were more difficult to run because they are much more sensitive than bacterial cells. Chemical and physical tests reveal the material contains no nitrogen, phosphorus, or sulfur. In fact, it is composed exclusively of carbon, hydrogen, and oxygen.

A chelate mechanism for the factor was suggested from bacterial studies when otherwise toxic concentrations of Mn^{++} permitted good growth in the presence of the GP factor. When mammalian cells were tested it was evident that concentrations of Mn^{++} that permitted growth were toxic when GP factor was added. A number of chelates (ethylenediaminetetraacetic acid, meconic acid, reductone, and kojic acid) were active in a manner similar to that obtained with GP factor in bacterial growth studies, yet in the mammalian system this could not be demonstrated.

It would appear that this factor has some common property that encourages early growth of some microorganisms and some mammalian cells, even though the mechanism appears to be different for each. With mammalian cells the factor demonstrates two of the properties reported for fetuin (*3*), the skim milk factor (*4*), and serine (*5*), in that it promotes early and firm attachment of cells to glass and partially substitutes for the growth-promoting materials present in serum (*6*).

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Determination of the Earth's Gravitational Field

Abstract. Brenner *et al.* have pointed out that spurious variations may be introduced into computation of satellite orbits by a combination of the use of osculating elements and a maldistribution of the observations. They suggest that this circumstance is the source of the eccentricity variations in the Vanguard I orbit which have been attributed to the third zonal harmonic. This criticism is based on a misunderstanding of the Vanguard orbit and tracking programs. The source materials for our study of the third zonal harmonic were not osculating elements, and the observations were in fact uniformly distributed around the Vanguard I orbit.

Brenner, Fulton, and Sherman (*1*) of the Stanford Research Institute have studied the variation of the osculating elements in a near satellite of the earth. They point out that the osculating elements show short-period variations, and that these short-period variations may masquerade as long-period terms of the type produced by higher harmonics in the earth's field, if the observations are not well distributed around the orbit. From this and certain criticisms of the distribution of Minitrack stations in the Vanguard network, they deduce that the third harmonic found by myself and my co-workers at the National Aeronautics and Space Administration (*2*) and by Kozai (*3*) at Harvard is spurious, and conclude that "evidence that the Earth's potential field has an odd harmonic is lacking."

This criticism is based on a misunderstanding of the Vanguard orbit and tracking programs. First, the orbital elements in our calculations are not osculating elements, as Brenner *et al.* assume; they are instead the constants of integration in a Hansen-type theory. Apart from the effects of drag and odd harmonics, these constants should show no variations. Brenner *et al.* advocate the replacement of the presumed osculating elements by another set of constants, in which the periodic variations

do not appear, as a means of eliminating the misleading effects to which they refer. This program has in fact already been carried out by Herget and Musen (*4*) and forms the basis for the Vanguard orbital calculations. Second, the distribution of the Minitrack stations is, as pointed out by the Stanford Research Institute group, concentrated along the 70th meridian. However, the location along the meridian does not imply a bias favoring a particular portion of the orbit, as they suggest. The rotation of the earth actually spreads the successive observations out along the orbit at very reasonable intervals of about 35 degrees.

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24 November 1959

Colors of All Hues from Binocular Mixing of Two Colors

Abstract. Land has recently studied the perception of colors resulting from appropriate mixtures of two colors or of one color and light from an incandescent lamp. In an "image situation," colors of all hues may result from such mixtures. The findings presented demonstrate that the mixing which Land accomplished by superimposing two projected images on a screen can be achieved when the two color separation images are presented simultaneously but separately to the two eyes.

The problem of binocular fusion of colors has interested investigators since Hecht's demonstration in 1928 that presenting red to one eye and green to the other led to a subjective sensation of yellow (*1*). Hurvich and Jameson (*2*) confirmed these results; it is today generally accepted that such fusion is readily obtainable in most subjects.

Land (*3, 4*) has recently considerably extended the early work of Fox and Hickey (*5*) and of Bernardi (*6*) on colors resulting from mixtures of two colors or of one color and light from an incandescent lamp. In the "image situation" (that is, a complex array of natural objects), colors of all hues may result from such mixtures. We decided to study the question of whether or not such mixing occurs when the two color separation images were presented simultaneously but separately to the two eyes.

Our experimental procedure followed Land's closely, deviating primarily in the technique of viewing. A complex scene was photographed on Kodak 35-mm direct positive film through various Kodak Wratten filters. Pairs of positive transparencies were selected in which the scale of grays was complete. The positives were then viewed in a Kodak stereoscopic viewer (however, the pictures used were not stereo pairs) with appropriate filters place in each half. Thus, in a typical experiment a scene was photographed through a Kodak Wratten filter No. 29 (red) and through a Kodak Wratten filter No. 58 (green). The black and white positive photographed through the red filter was placed on the left with a No. 29 filter and the positive photographed through the green filter was placed on the right with either a No. 58 filter or with no filter at all. A pair of crossed polarizing screens (Kodak Polascreen) placed on the brighter side was adjusted for optimum color. The brightness control of the viewer was then adjusted for maximum color saturation.

Under these conditions of binocular mixing, as full a range of colors was seen as had been obtained by projecting the two images on a screen, as in Land's experiments. If the filters were interchanged, "color reversal" occurred (that is, greens appeared as reds and vice versa).

The balancing of brightness in the two images is of critical importance since, otherwise, many subjects have great difficulty in fusing the colors, seeing predominantly with one eye. This effect varies from subject to subject, presumably because of varying degrees of retinal rivalry. Retinal rivalry probably also explains the fact that the perception of the colors may vary in time, in contrast to their constancy when projected and superimposed.

Under the conditions of this experiment, the perception of colors of all hues from two-color mixtures cannot be a purely retinal effect but must involve the interaction of higher centers.

Students of simple binocular color mixing have tended to explain their experiments in terms of color mixing at some level higher than the retina (*1*) or as the central "cancellation" of the hues not common to the two eyes, leaving the hue common to the two eyes to be perceived centrally (*2*). Another equally acceptable explanation is that the images in each eye initiate reflexes at higher levels whose efferent limbs modify the response of the opposite retina. Because of the possibility that this explanation is correct, it cannot be excluded that under some conditions the perception of colors of all hues from two-color mixture is a retinal effect, or that parallel mechanisms are present in the retina and higher centers (*7*).

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Interspecific Transformation of Neisseria by Culture Slime Containing Deoxyribonucleate

Abstract. Genetic change of *Neisseria meningitidis* is elicited by deoxyribonucleate preparations obtained from *N. sicca*. Such interspecific transformation is effected not only by deoxyribonucleate obtained from cells by conventional methods but also by crude deoxyribonucleate-containing slime which accumulates without experimental intervention in some cultures incubated for a period as short as 44 hours.

Deoxyribonucleates (DNA) of high molecular weight are recognized as determinants of bacterial heredity (*1*). Deoxyribonucleate which has been extracted from donor bacteria after lysis by added deoxycholate or other lytic agents, and extensively purified, elicits heritable change (transformation) when applied in minute quantities to suitable recipient bacteria. The genetic changes which have been studied affect numerous properties of the bacteria, including virulence, specificity of antigens, cellular and colonial morphology, resistance to various antibacterial agents, and capacity to utilize certain compounds for nutritional purposes (*1, 2*). Accumulated evidence led Hotchkiss (*1*) to the conclusion that the transforming DNA contains biologically specific entities operationally equivalent to bacterial genes.