the phenomenon seems to be very general. Similar recovery has been noted with several intensities of punishment, levels of food deprivation, and schedules of reinforcement, as well as with electrified grids rather than implanted electrodes.

The temporary increase in responding after the removal of punishment seems to involve some type of contrast effect. Previous studies have revealed that the addition of punishment during extinction (*1*) produced a reduction in responding and that the later removal of the punishment produced a temporary increase in responding which appeared to be a type of compensation for the behavioral reduction. This study (*3*) reveals that this same temporary increase follows the removal of the punishment, even though the punishment has ceased to be effective. It appears, therefore, that this compensatory increase in responding does not require that the behavior in question be suppressed at the time the punishment is removed.

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5 October 1959

Correction of Sample Absorption of Radioactivity

Abstract. In the method presented here for the correction of sample absorption of C^{14} activity, the only requirement is the availability of any C^{14}-labeled compound of sufficiently high specific activity to permit addition, in negligible mass, of a number of counts equal to or greater than that in unknown samples and with solubility characteristics that exclude preferential layering during drying of samples. The principle may be applied to liquid scintillation counting. Absorption curves are dispensed with, and the weights of the assayed samples need not be determined.

In a recent article, Hendler (*1*) has presented an elaborate new correction for self-absorption of C^{14} which is based on a variable absorption coefficient that has been found empirically to decrease hyperbolically with the weight of the sample. We have for some time employed (*2*) a method for correction due to sample absorption of C^{14} (and other weak β-emitters) that is extremely simple and requires no knowledge of the weights of samples, the variability of absorption with weight of sample, geometrical conditions, or other factors. The method requires only the counting of a duplicate sample to which is added, before drying, a known quantity of a standard solution of the C^{14}-labeled compound being assayed, the added C^{14} activity being *essentially carrier-free compared to the weight of the unknown sample.*

If U and S represent the number of microcuries of C^{14} present in the unknown sample and the added standard, respectively, A is the counting rate of the unknown sample and B is the counting rate of the duplicate unknown sample containing the added known quantity of S, then

$$cU = A$$

$$cU + cS = B$$

where c is the counting rate per microcurie of C^{14} under the particular conditions of assay and is the same for both samples. These equations permit determination of the C^{14} content of the unknown sample in absolute units (microcuries) if that of the standard is known. However, it is frequently necessary to know only the relative C^{14} contents of different unknown samples or the C^{14} content in terms of a standard. Then

$$U = [A/(B - A)]\,S$$

and unknown samples of varying and undetermined weights may readily be assayed relative to the standard and to each other without further correction for absorption in the samples.

To avoid random errors of small differences, the amount of S added should be at least as great as, and preferably much greater than, that expected in the unknown sample. For greater accuracy a third sample may be prepared in which the amount of added S is doubled.

It is not essential that the standard C^{14} compound be identical with that in the unknown sample if the two compounds do not sediment out differently during the plating procedure. This requirement is easily tested.

The method is also applicable to liquid scintillation counting with a single modification. Here, equal volumes of solvent, with and without standard, are added to duplicate samples of the unknown to provide for equal quenching in both samples.

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8 October 1959

A Growth-Stimulating Factor for an Epithelial Cell Line in a Reduced Serum Medium

Abstract. A concentrate obtained from heated solutions of glucose and phosphate demonstrated a stimulatory effect upon epithelial cells cultured in vitro. Survival, attachment to glass, and early cell division were influenced favorably.

A number of references have been made concerning the catalysis of biologically active substances in heated solutions containing glucose (*1*). The activity of this heat-catalyzed substance(s) has generally been described as one of growth stimulation or more rapid spore germination. All of the existing references reported upon results obtained with microorganisms, but the activity covered such a rather large group of bacteria, yeasts, and molds that it seemed worth while to see whether a similar result could be obtained in tissue cultures in which mammalian epithelial cells were the test system.

The problem was simplified somewhat because a concentrate of the active material was available from a previous study (*2*). This bacteriologically active concentrate was tested and was found to produce measurable decreases in the generation time of mammalian cells up to 8 days in culture. This effect was most pronounced when moderately small inocula were used and when the serum concentration was reduced below the optimum concentration.

The concentrate, designated GP factor, was isolated from a solution containing 150 gm of glucose, 20.1 gm of K_2HPO_4 and 9.9 gm of KH_2PO_4 in 1500 ml of demineralized water. The solution was autoclaved for 20 minutes at 15 lb pressure, cooled, and absorbed with 10 gm of acid-washed Norit A for 12 hours. The charcoal was then washed successively with water and methyl alcohol until the filtrate was colorless. The active factor was then eluted from the charcoal with a 40 : 60 pyridine-water solution. Vacuum distillation of this eluate yielded a dark brown, pyridine-free residue, which was resuspended in 20 ml of water and filter-sterilized for testing.

An inoculum of about 10,000 cells per milliliter was used in these tests and was taken from glass-grown cultures of HELA (original) cells. The cells were removed from the glass by scraping with a rubber policeman, trypsinized for even suspension, washed twice by centrifugation in basal salt solution, and then added to cultures containing varying amounts of horse serum. The inoculum size was determined by whole cell counts in a hemacytometer. The cells were examined at selected time

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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20 November 1959

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