Reports

Some Characteristics of a Continuously Propagating Cell Derived from Monkey Heart Tissue

The transformation of cells, in the course of cultivation of normal tissue, with the development of the property for unlimited growth, has long been known (*1*) and, in recent years, has been reported repeatedly. Such a continuously propagating cell line, derived from a culture of trypsinized heart tissue of an apparently normal cynomolgus monkey, has been under investigation in this laboratory, in part because of the desirability of developing an established cell line for propagation of virus for vaccines for human use (*2, 3*).

Because of the unresolved doubt concerning the possibility of inducing neoplasia by the injection of vaccines prepared from virus cultivated in continuously propagating cell lines, the use of such cells for this purpose has been avoided. Since the cell line to which reference is made here was of simian and not of human origin, it seemed that it could be tested more readily for neoplastic potential in the native animal from which it arose than could cells of human origin, and that the question could possibly be answered directly. The answer was sought in tests done in more than 200 rhesus and cynomolgus monkeys, inoculated since June 1956 with different quantities of cells administered via different routes. Thus far, there is no evidence of malignant neoplasia; however, in a number of instances, rather large (3 to 4 cm in diameter) localized tumors were induced by inocula containing from 1 million to 50 million cells injected subcutaneously. In some animals, palpably detectable growth of cells has resulted from smaller inocula. All tumors regressed completely after 2 weeks to 3 months, and none have been observed to return or to reappear in other locations. The absence of evidence of malignant effects in untreated monkeys is in contrast to the findings of Coriell and his associates (*4*) that, in weanling rats treated with x-rays and cortisone, the continuously propagating monkey heart cell line, given intraperitoneally, multiplied and caused death.

In monkeys in which tumors regressed, or in animals inoculated with sufficient quantities of whole or of lysed cells, cytotoxic antibody appeared in the serum; it was also observed that such monkeys were refractory to the formation of new tumors. These findings are not unlike those observed for a transplantable rat tumor (*5*), where cytotoxic antibody and immunity developed in rats in which tumors regressed.

It seemed important to study the relationship of this to other cells. This is being done by measurements of cytotoxic antibody by a technique that is similar in principle to a tissue-culture color-test used for measuring cytopathic effect of poliovirus (*6*). It is the usual serologic practice to test a serum for the extent to which it can be diluted before extinction of the particular property that is being measured; in this case, dilutions of serum would be tested against a constant concentration of metabolizing cells. In certain instances, the optimal concentration of cells may be difficult to select with sufficient accuracy, within a range that is sometimes critically narrow; therefore, there is some advantage to an alternative procedure in which the serum component is kept constant and the cytotoxic effect is determined upon a graded series of cell concentrations. Although the details to follow describe a procedure based on the latter scheme, the principles that apply are the same for measurements of cytotoxic activity in either dimension.

Into each of a series of 13 by 100 mm test tubes is placed 0.25 ml of the desired dilution of unheated test serum (usually 1: 4). Mixture 199 is used as diluent; to it is added 3 parts per 100 of 2.8 percent $NaHCO_3$ solution, 0.02 mg of phenol red per milliliter, and antibiotics in the following concentrations: penicillin, 200 units/ml; dihydrostreptomycin, 10 μg/ml; mycostatin (Squibb), 40 units/ml; and tetracycline, 10 μg/ml. To each tube containing test serum, or control substance, is then added 0.5 ml of one of a series of different cell concentrations, prepared by suspension in mixture 199, to which has been added $NaHCO_3$, antibiotics, and 10 parts per 100 of calf serum (Seitz-filtered and heated at 56°C for ½ hour).

The cell suspension is usually prepared from 6- to 7-day cultures maintained in continuous passage in a medium consisting of mixture 199 plus $NaHCO_3$, antibiotics, and 10 percent calf serum. The growing cells are detached from the glass surface of the flask by treatment with 0.25 percent trypsin solution (Difco 1:250 Trypsin), centrifuged at 500 rev/min for 5 minutes, and resuspended for cell count and adjustment to the required cell concentration. The range of cell concentrations used in the test is from 320,000 per 0.5 ml to 625 per 0.5 ml or less; however, the majority of tests have been done by using eight twofold steps from 80,000 to 625 cells per 0.5 ml. (When trypsinized tissue is used, rather than cultured cells, concentrations are expressed as dilutions of packed cells sedimented at 500 rev/min for 5 minutes; the range of dilution has been from 1:50 to 1:52,200, depending upon the degree of activity of the particular tissue suspension.)

After the serum-cell mixture is overlayed with 0.5 ml of heavy mineral oil, it is incubated at 36.5° to 37.0°C; the *p*H of the reaction mixture at the start is about 7.6, and upon overnight incubation, or even in a few hours, in tubes containing a sufficient number of actively metabolizing cells, the *p*H will fall to 6.8 and the phenol red will turn yellow. In tubes containing smaller numbers of cells, or cells that metabolize more slowly, the color change proceeds more or less slowly, with variations from red through orange and then yellow, indicating *p*H values that can be assigned by comparison with a stable set of standards; final readings are made at 7 days, or sometimes later, depending upon the purpose of the test.

Since the cytotoxic activity of serum is relative and is established on the basis of comparison with a control serum, the indications of trends are often evident from overnight incubation. When cells fail to metabolize, the *p*H is 8 or above and is identifiable by comparison with the color of medium without added cells. The *p*H's of individual tubes are recorded, and the numerical value for the highest number of cells, in the twofold cell-dilution series, that induces sufficient metabolic activity to result in *p*H of 7.4 is used for expressing quantitatively the degree of cytotoxic activity; the higher numbers reflect the greater degree of cytotoxicity.

Antisera from monkeys inoculated with the continuously propagating heart

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All technical papers and comments on them are published in this section. Manuscripts should be typed double-spaced and be submitted in duplicate. In length, they should be limited to the equivalent of 1200 words; this includes the space occupied by illustrative or tabular material, references and notes, and the author(s)' name(s) and affiliation(s). Illustrative material should be limited to one table *or* one figure. All explanatory notes, including acknowledgments and authorization for publication, and literature references are to be numbered consecutively, keyed into the text proper, and placed at the end of the article under the heading "References and Notes." For fuller details, see "Suggestions to Contributors" in *Science* 125, 16 (4 Jan. 1957).

cell exhibit cytotoxicity for this cell but exhibit little or no cytotoxicity for cells from primary cultures of monkey kidney or heart tissue. On the other hand, antisera from inoculated chicks are cytotoxic both for the continuously propagating cell line and for cultured cells from tissues of these organs. Thus, the common antigen or antigens between the continuously propagating cell line and the cells derived from freshly extirpated organs of the monkey is revealed by the chick; the monkey, however, reveals the existence of another antigen in the continuously propagating heart cell that is not present in cells from freshly removed organs of the monkey.

The antiserum for the continuous monkey heart cell line is also highly cytotoxic for the following continuously propagating human cell lines derived from both normal and neoplastic sources: human carcinoma (HeLa, HEP-2, KB); human embryo [intestine (Henle)]; human marrow (Detroit 6); human conjunctiva and liver (Chang); human heart (Girardi).

The cytotoxic effect of both monkey and chick antisera has also been demonstrated for trypsinized suspensions of cells from human tissues, both normal (tonsil, lung, kidney) and neoplastic (melanoma, Wilms' tumor); similar effects have been shown for suspensions of cells cultured from these tissues. Adsorption of antibody on monkey heart cell does not remove all of the cytotoxic antibody for human tonsil, and adsorption of the cytotoxic antibody upon suspensions of normal human tonsil, or monkey kidney, does not seem to remove the cytotoxic activity against the established monkey heart cell. Furthermore, studies of antibody-combining capacity, by cells and by cell extracts, also reveal the complexity of antigens involved.

It is apparent that considerably further study is required before any conclusions can be drawn. The purpose of this communication is to report a simple technique for measurement of cytotoxic antibody by means of which the relationship among continuously propagating cells may be investigated and, through this, the question of the use in man of vaccines prepared from such cells can be dealt with further.

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References and Notes

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3. We wish to acknowledge collaboration with L. J. Lewis, Francis Yurochko, Donald Wegemer, and Louise Boccella in related aspects of these studies that have not yet been published. This work was aided by a grant from the National Foundation for Infantile Paralysis.
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8 November 1957

Rate as Response Probability in Discrimination Learning

It has been argued that rate of responding is the only appropriate datum to a formulation of behavioral change in terms of response probability (*1*). It has also been shown that, under certain restrictions, rate is mathematically equivalent to response probability (*2*). The experiments reported in this paper were designed to evaluate response rate as it corresponds to specific probabilistic predictions.

In discrimination, the organism comes to respond primarily to one set of stimuli (S) which are the occasion for reinforcement, or reward, and not to another set (S') for which responding is never reinforced. This study is concerned with the forms of S and S' response curves when the probabilities of sampling, or perceiving, stimulus elements in S and S', θ_1 and θ_2, respectively, vary from equality to a considerable inequality. Response frequency is known to be quite sensitive to differences in the manner of reinforcement. Consequently, two conditions of reinforcement known to produce marked differences in response rate were employed to determine the extent to which the forms of the curves predicted on the basis of stimulus control are distorted or overriden by the effects of such reinforcement contingencies.

From a discrimination model (*3*) developed from the Estes-Burke statistical learning theory (*4*), one predicts that the S and S' response curves approach their asymptotes monotonically when the sampling probabilities or θ values are equal. When θ_1 is larger than θ_2, the S curve accelerates more rapidly than is the case in the equal θ condition, and the S' curve may increase to a "peak" before it declines to a lower asymptote.

In experimental groups I and III, θ values were contrived to be equal through the expedient of setting an equal number of experimentally manipulated stimulus elements in S and S'. In groups II and IV, six times as many elements were assigned to S' as to S; hence, for these groups, $\theta_2 = \theta_1/6$. Five subjects were run individually in each of the four groups. The subject was required to pull a Lindsley manipulandum (*5*) for points on a counter as a series of patterns of lights were presented to him. Each pattern, composed of subsets of ten jewel lights mounted in two rows of five lights each, was presented for 1 minute. The subject was instructed to try for a maximum score on the counter at the same time he was trying to determine the principal defining S patterns. The only other room illumination was supplied by a blue 7-watt bulb mounted above the counter: white noise was piped in through a speaker and headphones for masking purposes.

Groups I and II were placed on a 10/1 variable-ratio schedule of reinforcement—that is, subjects in these groups were awarded one point for every tenth response, on the average, made in the presence of S patterns. Figure 1 shows the mean frequency of response per stimulus pattern. In the second experiment, subjects in groups III and IV were placed on a 30-sec fixed-interval schedule of reinforcement—that is, they were awarded a point for the first response made after each 30 sec interval during S presentations. Only 25 S and 25 S' patterns were presented in these latter groups, as opposed to 30 S and 30 S' presentations in the ratio groups. As is shown in Fig. 2, the over-all mean response rates are considerably lower in the interval groups. This is in agreement with previous studies of the effects of schedules of reinforcement upon rate of response. It should be noted that, despite the large differences in rates obtained under the different schedules, the predicted ordinal positions of the curves are invariant and as predicted insofar as the more difficult discriminations are not far advanced. The S' curves under

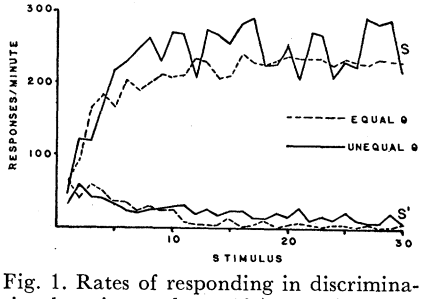

Fig. 1. Rates of responding in discrimination learning under a 10/1 variable ratio schedule of reinforcement.

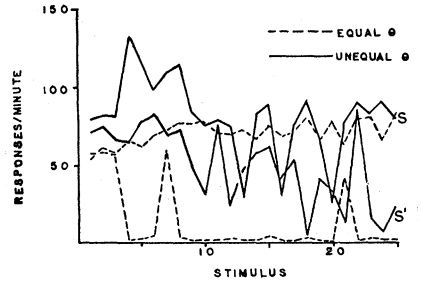

Fig. 2. Rates of responding in discrimination learning under a 30-sec fixed-interval schedule of reinforcement.