riched medium, which results in a longer lag before resumption of growth in minimal medium, the proportion of auxotrophs among the survivors of penicillin treatment was quite low. Presumably under these conditions the prototrophs resume growth less uniformly. The present method may therefore not lend itself to the selection of a wide variety of mutants in a single experiment (*8*).

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Sequential Effects of Punishment

Abstract. Punishment is found to produce a large reduction in reinforced responses when it is initially introduced. Continued exposure to punishment, however, results in substantial recovery within each hour of exposure as well as from day to day. A compensatory increase in responding occurs after the removal of punishment, even after the punishment has ceased to be effective.

The present report is concerned with the effects of punishment on behavior that is simultaneously being maintained by positive reinforcement. Previous studies (*1*) have indicated that responding is reduced so long as the punishment is in effect. The present findings reveal that the degree of suppression varies markedly during the course of the punishment process. White Carneaux pigeons, maintained at 80-percent of the weight they attained when allowed to feed ad libitum, were reinforced for 1 hour per day for responding (pecking) at an illuminated disc in accordance with a 1-minute variable-interval schedule of food reinforcement. Under this schedule, the response produces food reinforcement at varying time intervals, the average of which is 1 minute. This reinforcement procedure produces a fairly stable and uniform rate of responding which serves as a base line for evaluating the effects of punishment. This punishment was delivered immediately after every response and consisted of a brief electric shock delivered through implanted electrodes (*2*).

Figure 1 shows the effect of the addition of punishment for 23 days to the food-reinforced responses of one subject. The punishment used here is a 30-v, 60-cy/sec shock of 30-msec duration delivered through 10,000-ohms resistance in series with the subject. It can be seen that responding is reduced immediately by the initial addition of the punishment. On succeeding days the number of responses gradually increases, and recovery is complete after several days. At that time, the number of responses during punishment is equal to or greater than the number before punishment was introduced. It can be seen that, when the punishment is removed, responding increases for the first 3 days and then returns to a level approximating the prepunishment performance. It may be noted that the variable-interval schedule employed permitted the animal to receive as many food reinforcements during the punishment period as during periods without punishment as long as a low trickle of responses was made. The changes noted above of (i) a day-by-day recovery from the initial effects of punishment and (ii) a temporary increase in responding upon the elimination of punishment have both been replicated with several other subjects.

Recovery from the effects of punishment occurs not only from day to day but also within each 1-hour session. Figure 2 shows the actual cumulative response record for a different subject under more severe punishment—a shock of 10-ma intensity. Before punishment (Fig. 2, top) the rate of responding is fairly uniform throughout the hour, at about 110 responses per minute. Under punishment, however (Fig. 2, bottom), the rate of responding shows a gradual recovery throughout the hour. In the first few minutes of the punishment period, the rate of response is essentially zero, but by the end of the hour, the rate of response stabilizes at about 15 responses per minute. The absence of complete recovery from punishment here is attributable to the greater intensity of the shock used. This response record was obtained after 20 days under punishment and represents a fairly stable state. The responses show an orderly increase throughout the hour, with no increase in variability such as is generally assumed to accompany punishment. The absence of such variability is in large part attributable to the corresponding lack of variability in the shock intensity, a nonvariability achieved through the use of implanted electrodes rather than the usual electrified grid. This recovery from the initial effects of punishment within each session has characterized the behavior of all of the 14 other subjects studied, although the degree of recovery may be somewhat more or less than that seen in Fig. 2. It may be noted that this recovery does not seem to be attributable to any local tissue changes, since recovery continued when the locus of the electrodes was changed during the recovery process. Rather,

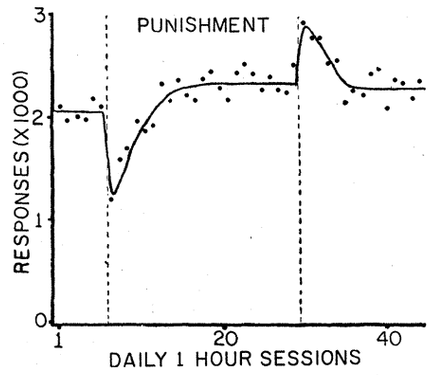

Fig. 1. Effect of the addition and removal of punishment upon the food-reinforced responses of one subject. The punishment was a brief electric shock which followed every response on the days between those represented by the vertical dashed lines. Food reinforcement was produced according to a variable-interval schedule with a mean of 1 minute on all days.

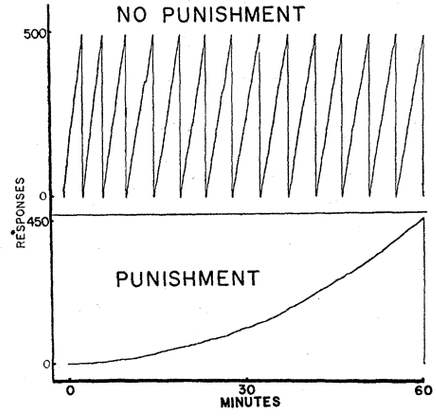

Fig. 2. (Top) Cumulative record of the food-reinforced responses of one subject prior to the addition of punishment. The food reinforcement (not indicated on the record) is produced at variable intervals, the average of which is 1 minute. The vertical lines represent the resetting of the recorder pen. (Bottom) A cumulative record of the responses for the same subject during punishment. The same schedule of food reinforcement prevails, but punishment in the form of a brief electric shock is produced by every response.

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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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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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