advantages. When the problem involved is that of estimating low concentrations of AER cells (for example, 0.01 percent) in a predominantly aer population, the advantages of the selective lactate agar technique over TTC overlay become apparent (*6*).

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11 February 1957

Catabolism of Hexuronic Acids by Erwinia and Aerobacter

Only casual information is presently available on the metabolism of hexuronic acids by microorganisms (*1*); somewhat greater development of this field is evident with plant (*2*) and animal (*3*) materials. Our interest in this subject stems from an earlier study of the pectinolytic action of *Erwinia* soft-rot bacteria in which it was shown that pectin is broken down to galacturonic acid (*4*) and that the uronate is further catabolized to a mixture of end-products (*5*). For the past few years we have sought the initial step in the breakdown of galacturonate. We wish now to report that our investigations (*6*) indicate that the first detectable step in the catabolism of D-galacturonate and D-glucuronate by cell-free extracts of *Erwinia carotovora* and *Aerobacter cloacae* is a reduction, with either reduced triphosphopyridine nucleotide (TPNH) or reduced diphosphopyridine nucleotide (DPNH), to the corresponding hexonic acid, namely, L-galactonate and L-gulonate.

These reductions are carried out by substrate-induced enzymes. Glucose-grown intact cells oxidize glucose at once in manometric experiments, but act on galacturonate and glucuronate only after an induction period. On the other hand, galacturonate-grown intact cells of *Erwinia carotovora* respire galacturonate at once or after a brief induction phase; glucuronate is used very slowly. These galacturonate-grown cells are adapted at the same time to the oxidation of L-galactonate, but not to D-galactonate, D- or L-galactose, mucate, D- or L-lyxose, D-xylitol, L-arabitol, L-ascorbate, L-arabinose, or D-fucose. This sequential induction pattern suggests L-galactonate as a possible intermediate and reduces the likelihood that some often-suggested schemes (*1*) for galacturonic acid catabolism are operative in *E. carotovora*.

Glucuronate-grown intact cells of *Erwinia carotovora* oxidize glucuronate and galacturonate after a short induction phase; galacturonate- and glucuronate-grown cells of *Aerobacter cloacae* oxidize both uronic acids rapidly without a lag period; the last mentioned cells are sequentially induced to oxidize L-gulonate.

Cell-free extracts were prepared from both *Erwinia* and *Aerobacter* that had been grown on either galacturonate or glucuronate, by sonic oscillation and centrifugation. The supernatant was dialyzed overnight at 4°C against 0.05*M tris*-HCl buffer of *p*H 7.2 and centrifuged at 100,000 *g* for 90 minutes; the clear solution was used. These preparations contain an enzyme that catalyzes the reduction of both galacturonate and glucuronate by TPNH or DPNH. No reduction of D-mannuronate has ever been observed. The enzyme is provisionally named uronic reductase. When a preparation from galacturonate-grown *Erwinia* is used, DPNH reacts slightly faster with galacturonate than it does with glucuronate; when TPNH is the reductant, the reaction with glucuronate is about 5 times faster than it is with galacturonate under the conditions of our experiments. This difference is less distinct with preparations from cells of *Erwinia* grown on glucuronate, or *Aerobacter* grown on either uronate. The activity of the uronic reductase is very weak in extracts from glucose-grown cells of either species.

The end-products of the reduction of the uronates by both bacterial species, with either DPNH or TPNH, are nonreducing, nonlactonized acids: L-galactonate from galacturonate, and L-gulonate from glucuronate. By boiling for 5 minutes with 1*N* HCl, the acids are converted into the corresponding lactones which, on paper chromatograms, behave in every respect like L-galactono-γ-lactone after galacturonate reduction, or L-gulono-γ-lactone after glucuronate reduction. It is highly unlikely that the reaction proceeds by lactonization of the uronic acid followed by reduction to the corresponding L-hexonolactone; this opinion is based on (i) the failure of extracts of *Aerobacter cloacae* to reduce D-glucurono-γ-lactone with DPNH or TPNH, and (ii) the actual accumulation of the free L-hexonic acids taken together with the observed lack of delactonizing enzyme activity in the extracts for either of the L-hexono-γ-lactones.

The recent report by Payne (*1*) regarding the ability of dried cells of *Serratia marcescens* to form from glucuronic acid a substance tentatively identified as a "1,6-ester linked dihexuronic acid," prompted an examination by us of uronic reductase activity in that microorganism: an enzyme preparation from glucuronate-grown *Serratia* reduces glucuronate with TPNH or, somewhat more slowly, with DPNH. The relationship of the aforementioned reactions to the system described by Isherwood *et al.* (*2*) in pea mitochondria (which reduces the methyl ester of galacturonate, but not galacturonate, to L-galactono-γ-lactone) is under investigation, as are extensions of this general reaction to some of the less common uronic acids.

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30 January 1957

Effects of Pentobarbital on Intermittently Reinforced Behavior

The behavioral effects of drugs have recently (*1–5*) been measured in terms of changes in the stable temporal patterns of responding that occur when behavior is reinforced intermittently. It has been shown that drugs may influence both the average rate and the pattern of emission of responses (*4*). The latter effects are of particular interest, since these patterns probably represent the operation of more subtle behavioral processes than are reflected in the average rate of response. In this report, a new measure of the temporal pattern of responding is used in order to compare the effects of a drug on temporal patterning with the effects on rate of response.

One form of intermittent reinforcement is designated as a fixed-interval

schedule. In this procedure, the first occurrence of the arbitrarily selected response after a fixed period of time makes the reinforcement (for example, food) accessible to the experimental subject. Responses that occur before the fixed time has elapsed have no explicitly arranged consequence. Animals show a characteristic pattern of response when reinforcements are delivered on a fixed-interval schedule (*6*). For example, if the size of the interval is 15 minutes, then there is usually no responding during the first 5 to 8 minutes of each interval. After this initial pause, the animal begins to respond, at first slowly and then more rapidly; the highest rates occur just prior to reinforcement. This pattern of responding is the most stable and most interesting feature of fixed-interval performance. Inasmuch as this highly reproducible progression of response rates is correlated with the passage of time, the performance can be viewed as a temporal discrimination.

The behavior generated by the fixed-interval schedule has been used as a base line for the study of sodium pentobarbital and has shown sensitivity in terms of changes in both average rate and temporal pattern of responding (*2, 5*). In the present experiment (*7*), measurements were taken of the changes that occurred in the fixed-interval performance when subanesthetic dosages of sodium pentobarbital were administered. The experimental animals were two food-deprived, adult, male, White Carneau pigeons. The response was the pecking of a Plexiglas disk, and reinforcement was brief (4 seconds) access to grain. Prior to drug administration, these pigeons were trained on a 15-minute, fixed-interval schedule of reinforcement for about 30 daily experimental sessions.

Each pigeon spent 8 hours a day in a sound- and light-insulated experimental chamber. Reinforcements were delivered on the 15-minute, fixed-interval schedule. After each reinforcement, all illumination in the chamber was extinguished for a period of 5 minutes. The number of key-pecks during this "time-out" was negligible. Each 15-minute interval is considered to begin at the termination of a time-out.

Sodium pentobarbital was injected intramuscularly during the second or third hour of experimental sessions. Drug administrations always took place immediately after a reinforcement; this left 4 to 5 minutes of time-out before the next interval began. Sessions in which there were injections were separated by at least one complete experimental session in which there was no injection. Dosages of 1, 2, 3, and 4 mg of sodium pentobarbital were each administered six times per pigeon. The various dosages were not given in any systematic order.

The top part of Fig. 1 shows the combined results for both pigeons for all injections. The number of responses per 15-minute interval for 12 consecutive intervals is obtained. Six of the 12 intervals are prior to injection and six follow. These numbers are then averaged, interval by interval, for each dosage of pentobarbital. Each curve, therefore, presents average data for 12 administrations (six for each pigeon) of each dosage.

The graded effect of pentobarbital can be seen in the first postinjection interval. In this interval the reduction in average number of responses is a function of the size of the dosage. One milligram does not seem to cause a substantial decrease in response-output. Beginning with the second postinjection interval, there are clear instances of increased responding as a result of administrations of sodium pentobarbital. The magnitude of this "excitatory" effect does not appear to be monotonically related to dosage. There is some indication that the time at which the maximal increase occurs depends on the dosage and that it is later with larger doses.

The number of responses per interval measures the animal's rate of response in successive 15-minute periods. It does not reflect directly the changes that occur in the temporal pattern of responding within single intervals after drug administration. The latter aspect of the drug effect is presented in the bottom part of Fig. 1. The data presented in both parts of Fig. 1 were obtained from the same sessions. The ordinate of the bottom part of Fig. 1 is a measure of the pattern of responding characteristic of the fixed-interval performance. The quarter-life of responses is the time taken, in any one interval, for the first one-fourth of the

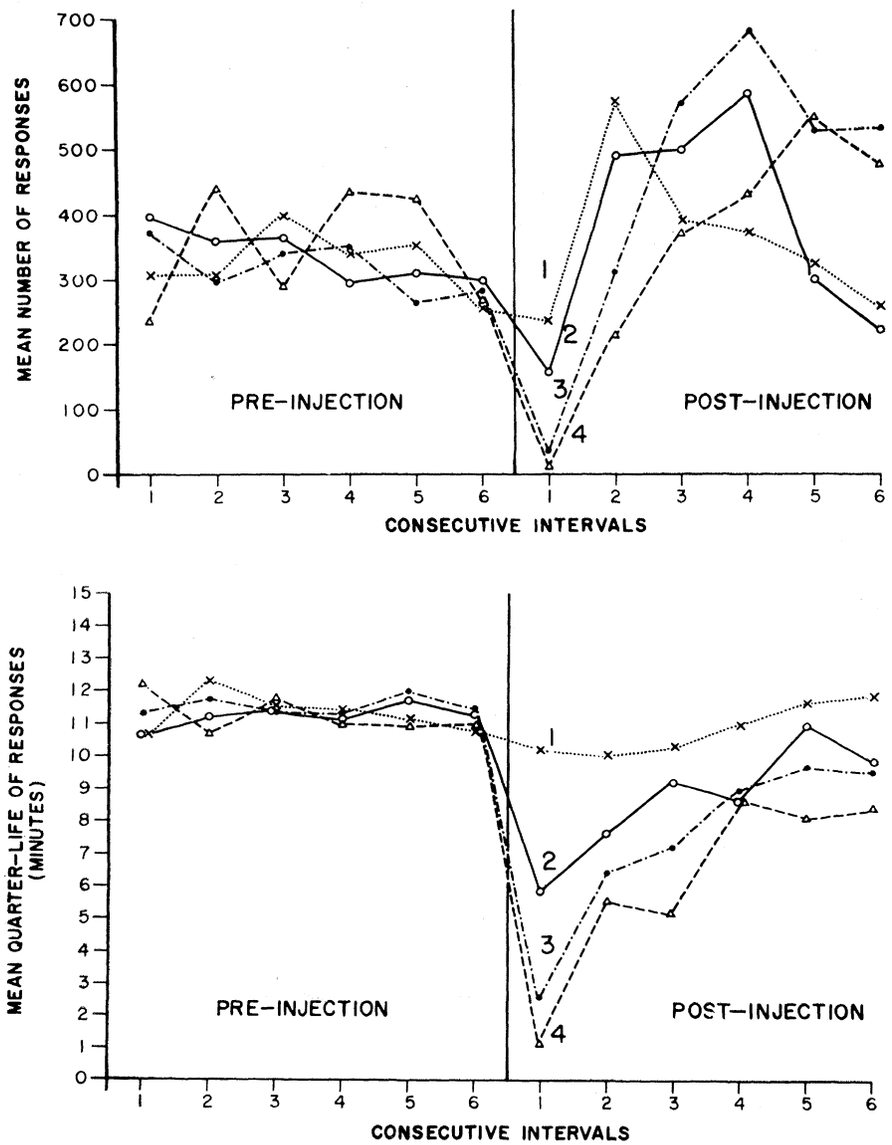

Fig. 1. (Top) Average number of responses per 15-minute interval for 12 consecutive intervals. Each point represents the average of 12 determinations. Numbers on the curves refer to dosages in milligrams of sodium pentobarbital. (Bottom) Average quarter-life for the same 12 consecutive intervals (see text for explanation of ordinate).

total number of responses in that interval to be emitted. For example, if the response rate in some interval were constant, then the quarter-life would be simply one-fourth of the duration of the interval. That is to say, when the rate is constant, one-fourth of the responses will occur in one-fourth of the time. If the responding within an interval has positive acceleration, then the quarter-life will be greater than one-fourth of the interval. On the other hand, if the rate should show a decline within an interval, then the quarter-life will fall below one-fourth.

The quarter-life prior to injection tends to lie between 11 and 12 minutes. This means that about four-fifths of the 15-minute fixed interval has elapsed when the first one-fourth of the responses in the interval have been emitted. The high value of the quarter-life expresses the rate increase that is characteristic of fixed-interval performance.

In the first interval following injection, the average quarter-life falls increasingly further below the base-line value, for 2, 3, and 4 mg of pentobarbital, respectively. This change reflects the loss of the characteristic rate increase within the fixed interval. In the case of 3 and 4 mg of pentobarbital, the quarter-life has fallen below 3¾ minutes. This indicates that the responding in these instances shows negative, rather than the customary positive, acceleration.

The time course of the drug effect on the quarter-life is seen in the consecutive postinjection intervals. By the sixth postinjection interval, the quarter-life has almost returned to the base-line value. The effect of sodium pentobarbital, as measured by the quarter-life, is a change in the characteristic pattern of responses associated with the fixed-interval schedule. The magnitude of the change varies directly with the size of the dose and dissipates gradually in time.

The drug effect appears to be analyzable into two components: a depressive effect and a loss of the positive acceleration in responding within the 15-minute interval. The increase in responding, shown in the top part of Fig. 1, is probably a consequence of the change in the temporal pattern of responding within intervals. As the depressive effect disappears, the absence of positive acceleration produces responding that occurs throughout, rather than at the end of, the 15-minute interval, thus increasing the over-all output of responses. The fact that the depressive effect (Fig. 1, top) disappears more rapidly than the loss of discrimination (Fig. 1, bottom) probably accounts for at least some of the increase in responding.

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25 February 1957

On the Distinction between the Effects of Agents on Active and Passive Transport of Ions

Several recent studies (*1–3*) designed to distinguish between the effects of pharmacologic and other agents on the active and passive components of ionic flux have brought out the difficulties of posing this question within the framework of current definitions of active transport. A brief analysis of the assumptions on which an unambiguous distinction of this kind can be founded seems, therefore, very desirable.

The ability to separate the effects of an agent on the two components of flux hinges, of course, on an experimentally unequivocal distinction between the active or passive components themselves. However, such a satisfactory quantitative distinction does not exist at present, and one is limited to a distinction based on the thermodynamic definition of active transport as transport against an electrochemical potential gradient. This definition was given by Rosenberg (*4*). The principal objection to this formulation is that it defines active transport in terms of a *net*—that is, a necessarily unidirectional—active flux. It specifically excludes metabolically linked transport (i) that is codirectional with and in excess of that expected from the electrochemical potential gradient and (ii) that is opposing but incompletely compensating the flux resulting from the gradient. One would intuitively wish to include both of these. The definition contains nothing to justify an interpretation of the net flux as a difference between two "active" components in the two directions, as has been suggested. In addition, it encompasses transport processes that require a supply of energy but do not derive it from metabolic reactions—for example, those leading to a Donnan equilibrium.

It may seem that one could define active transport as transport specifically coupled with metabolic reactions, without reference to the direction of the resulting net flux. However, in the absence of a precise knowledge of the mechanisms involved or of a possibility of measuring "passive" permeability coefficients in the *undisturbed* living system, such a definition does not prove experimentally meaningful. Numerous attempts have been made to arrive at a value for the thus defined "passive" term through the use of metabolic inhibitors. That it is, however, impossible in principle to make an unequivocal distinction between "active" and "passive" or even "metabolically dependent" and "metabolically independent" components of flux *solely* on the basis of experiments with metabolic inhibitors can be seen from the following considerations.

According to the most general definition, the isotopically measured flux in either direction would be expressed as a sum of a "passive" and an "active" term. The passive term should in principle be given by the product of a permeability coefficient, which is determined by the properties of the cell membrane and the activity of the ion on the side from which the flux originates. In the absence of evidence to the contrary —and such evidence is unobtainable without a clear-cut identification of the "active" term—the permeability coefficient must be assumed to depend on the state of metabolism as well as on ionic activity. Any metabolic inhibition must therefore be assumed to alter the permeability coefficient by an unknown amount and in an unknown direction. Thus one may not regard its measured value even as a meaningful extremum, making a distinction between the "active" and "passive" terms on this basis impossible.

More difficult to foresee is the impossibility of distinguishing by this approach between the components of flux which *are* and those which *are not* dependent on metabolism. We may represent the total (measured) flux by an equation such as

$$f_i = p^o\pi(m)\,a_o + f_i^*(m) \qquad (1)$$

where f_i is the total inward flux, p^o the value of the permeability coefficient in the complete absence of metabolism but with all other independent variables returning their values, $\pi\ (m)$ an unknown function describing its dependence on the rates of metabolic reactions, a_o the activity of the ion on the outside, and $f_i^*(m)$ the unknown active transport term. Even if perfect metabolic inhibition were achieved, there is no means of ruling out any effect of the inhibitor on the membranes, which itself is independent of metabolism, so that again the measured value $p^{o\prime}$ may in no way reflect even an extreme value of p^o. In other