The resting potential of the axons intracellularly perfused with solution rich in sodium was measured as follows. A saturated KCl-calomel electrode (Beckman) was connected to a long glass capillary (0.13 to 0.18 mm in diameter) filled with 0.6*M* KCl solution. (The electrical resistance of the electrode was 0.6 to 1 megohm.) First, the recording tip of the electrode was immersed in the surrounding fluid medium; then, it was introduced into the interior of the axon in place of the platinum electrode (Fig. 1). The d-c potential difference across the axon membrane, as determined between the same electrode in the two positions, was about 45 mv (range: 42 to 50 mv).

The resting potential is insensitive (*4*) to a large change in the sodium-potassium ratio in the perfusion fluid (Fig. 2). Within about 4 minutes after the onset of perfusion with a sodium-rich solution, both the resting and action potentials became stationary. When the axon was then perfused with a sodium-free, potassium glutamate solution, there was an immediate increase in the amplitude of the action potential, a reflection of the effect of the internal sodium ion. There was no detectable change in the resting potential during the period of rapid increase in the action potential. When the perfusion fluid was switched back to the original sodium-rich solution, the resting potential remained practically unchanged (within approximately 3 mv). The gradual loss of excitability in the terminal period of perfusion was accompanied by a decline in the resting potential. Since sodium is less favorable than potassium for maintenance of the normal macromolecular state of the membrane (*5*), both the action and resting potentials tend to deteriorate sooner during perfusion with sodium-rich media.

The amplitudes of the action potentials obtained in our present experiments agree well with those reported earlier. The value stated by Tasaki and Takenaka (fig. 2 in reference *6*) in axons with 0.3*M* sodium on both sides of the membrane is about 80 mv. The value reported by Tasaki and Luxoro (*1*) on *Dosidicus* axons under similar conditions is about 70 mv (or slightly more). The value obtained by Hodgkin and Chandler (*2*) under slightly different experimental conditions is about 65 mv. We believe that the differences among these values are very small in view of the differences in the experimental procedures and in the external divalent cation concentrations. We conclude, therefore, that there is no significant error in our previous measurements of action potentials in axons perfused with sodium-rich media with recording electrodes having a relatively high resistance.

In the previous experiments on *Dosidicus* axons, precautions were taken to avoid errors in recording, either by (i) anesthetizing the unperfused portions of the axon with isotonic $MgSO_4$ solution, or (ii) eliminating the outlet cannula completely. A precaution taken by Tasaki and Takenaka (*6*) was to record the potential variation ($v$) in the outlet cannula together with the potential ($V$) in the middle of the perfusion zone; at the moment when $d(v-V)/dt$ vanishes, the capacitative flow of current through the wall of recording electrodes is close to zero and hence the error arising from the current is negligible.

The resting potentials recorded by Hodgkin and Chandler (*2*) vary between 80 and 110 mv and are very different from the values obtained by our technique (about 50 mv). Since the overshoot is defined as the difference between the action-potential amplitude and the resting potential, our value of the overshoot is much greater than the values reported elsewhere (*2*), and does not agree with the Nernst equation applied to sodium ion. We believe that the origin of the discrepancy in the overshoot values is not in the measurement of the action-potential amplitude but in the procedure for determining the resting potential.

The potential difference (electric) across the resting axon membrane cannot be rigorously defined from the standpoint of thermodynamics (*7*). For example, it is extremely difficult to evaluate the liquid-junction potentials between the internal and external solutions and the recording electrode, especially in the presence of divalent cations. In electrophysiology, the "resting potential" (or resting emf) can only be defined operationally; hence, the observed value of the resting potential may vary with the procedure used for the measurement. The procedure adopted elsewhere (*2*) (agar bridges and electrodes with unplatinized platinum) is very different from that in the present observations. In addition to the problems arising from salt bridges, it is well known that the potential of a platinum electrode changes drastically with slight variations in the electric current, in the $O_2$ tension, and in the concentration of trace polyvalent cations (*8*, p. 110). This latter factor may account for wide variation in the values of resting potential with such irreversible electrodes. Therefore, it is not very surprising that the results reported are very different.

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## Effect of Gamma Radiation on Dietary and Hormonal Induction of Enzymes in Rat Liver

Abstract. *The dietary induction of serine dehydrase, produced by the oral intubation of hydrolyzed casein to protein-depleted rats, is markedly inhibited by doses of $\gamma$-radiation of 400 roentgens or higher provided the irradiation is given within an hour after the initial dose of casein. If the irradiation is delayed until 7 hours after the initial dose of casein, induction is not inhibited. In contrast, the cortisone induction of tyrosine $\alpha$-ketoglutarate transaminase is not inhibited by doses of $\gamma$-radiation up to 3200 roentgens; in some instances hormonal induction of this enzyme appears to be enhanced by irradiation.*

Previous studies (*1*) showed the effects of the antibiotic actinomycin D, as well as of the antimetabolite fluoroorotic acid, on the dietary induction of serine dehydrase. When actinomycin was administered at the beginning of

Table 1. Effect of $\gamma$-radiation on the dietary induction of serine dehydrase in rat liver. Enzyme units are expressed in micromoles per hour per gram of liver ± standard error of the mean. Numbers in parentheses denote the number of rats in each group. Casein hydrolyzate (1 g in 3 ml of $H_2O$) was intubated at zero hour and 6 hours, and the animals were killed at 0, 6, and 12 hours. Gamma radiation in the dosages shown was administered from a $Co^{60}$ source as detailed in the text. Animals killed at 6 hours received radiation between the 1- and 2-hour points, and rats killed at 12 hours received radiation between 7 and 8 hours.

| Time animals were killed (hr after radiation) | Enzyme units (micromoles per hour per gram of liver) |
|---|---|
| 0 Time (control) (8) | 50 ± 30 |
| 6-hr control (5) | 720 ± 140 |
| 6 hr + 400 r (6) | 270 ± 85 |
| 6 hr + 1600 r (4) | 189 ± 35 |
| 12-hr control (5) | 1420 ± 350 |
| 12 hr + 1600 r (4) | 1565 ± 150 |

the casein administration, a complete inhibition of enzyme induction occurred. However, when the administration of the antibiotic was delayed until 12 hours after initiation of the intubations, no inhibition of enzyme induction occurred, although the antibiotic puromycin completely curtailed further enzyme induction after the 12-hour point. These data indicate that the failure of actinomycin to act at this time is not due to the formation of a precursor protein or a similar mechanism. Furthermore, the effects of actinomycin were mimicked by the antimetabolite fluoroorotic acid, which, in vivo, is largely converted to fluorouridylic acid (*2*), an intermediate thought to be incorporated into RNA as a fluorinated pyrimidine nucleotide, producing a "false" RNA template. These data were interpreted as indicating that the messenger RNA (mRNA) template for serine dehydrase was formed shortly after the initial intubation of casein, then stabilized after 2 to 3 hours (*3*), and thus no longer required further renewal. In this stable state, the expression of the mRNA template would be resistant to the effects of the administration of actinomycin and fluoroorotic acid.

In view of the recent data demonstrating that ultraviolet (*4*) and gamma irradiation (*5*) inhibit enzyme induction in bacterial systems, and the considerable data on the effect of x-irradiation on the process of liver regeneration (*6*), studies were undertaken to determine the effect of high doses of $\gamma$-radiation on the dietary induction of serine dehydrase during the periods when the induction was either sensitive or resistant to actinomycin D. Additional studies were also performed involving the effect of $\gamma$-radiation on the induction of tyrosine $\alpha$-ketoglutarate transaminase produced by the administration of corticosteroids. The induction of this enzyme is also sensitive to the action of actinomycin (*7*).

The conditions for the dietary induction of serine dehydrase were essentially the same as those already described (*1*). Male Holtzman rats weighing 140 to 160 g were housed in the animal quarters of the Biology Division at the Argonne National Laboratory and maintained for 1 week on protein-free pellets (obtained from General Biochemicals Inc.). At the end of this period the rats were fasted overnight and the intubation with 1 g of casein hydrolyzate was begun at 6 a.m. the following morning. Rats in one group were irradiated for 1 hour with varying doses of $\gamma$-rays from a $Co^{60}$ source and were killed 6 hours later. Rats in another group were not irradiated, but were intubated at 0 or 6 hours, or both, and were killed at 6 or 12 hours. A third set of rats was intubated at 0 and 6 hours and irradiated for 1 hour, beginning at the 7th hour after the initial intubation. In this way the sensitivity of the dietary induction of serine dehydrase to $\gamma$-radiation was tested during a period of actinomycin D sensitivity (1 hour after the initial casein intubation) as well as actinomycin D resistance (7 hours after initial casein administration). The results are seen in Table 1. These data show that a dose of 400 r given at 1 hour causes an inhibition of enzyme induction greater than 50 percent, whereas 1600 r inhibits more than 80 percent of the control induction. However, 1600 r given at the 7-hour point has no effect on further enzyme induction. In fact, other studies (*8*) have indicated that even 6400 r given at a period of resistance to actinomycin D results in no inhibition of serine dehydrase induction. Thus, with this enzyme, whose synthesis appears to be controlled primarily by dietary amino acids (*1, 9*), periods of induction sensitive to actinomycin D are also sensitive to the action of total-body $\gamma$-radiation. Moreover, if the irradiation is given during a period in which the induction is resistant to actinomycin, the synthesis of serine dehydrase is also unaffected even by relatively large doses of $\gamma$-radiation.

In view of these findings the induction of several other hepatic enzymes was tested. Ornithine-$\delta$-transaminase, also induced by dietary means, responded in a fashion similar to serine dehydrase (*8*). Tyrosine transaminase, on the other hand, when induced by hydrocortisone (Table 2), gave a quite different picture after irradiation of the rats. Previous studies (*7, 10*) had shown that the induction of this enzyme was sensitive to actinomycin D up to 1 hour after corticosteroid administration (*11*) and that the induction was accompanied by a synthesis of RNA (*10*). Therefore, $\gamma$-radiation was given during the first hour after the administration of hydrocortisone. The control group (nonirradiated + hydrocortisone) showed a two- to threefold induction of the enzyme; however, the most striking fact was that doses of radiation ranging from 50 to 3200 r not only did not inhibit enzyme induction, but actually appeared to enhance it. The enzyme activity in adrenalectomized rats that had received 3200 r was almost seven times greater than that in the controls at zero time. Adrenalectomized rats were used to minimize any adrenal-mediated stress reaction which might result from the radiation.

Table 2. Effect of $\gamma$-radiation on the corticosteroid induction of tyrosine transaminase in rat liver. Enzyme units are expressed as micromoles of *p*-hydroxyphenylpyruvate per hour per gram of liver ± standard error of the mean. Numbers in parentheses denote the number of animals in each group. Adrenalectomized rats maintained on 1 percent NaCl in their drinking water for 6 days after the operation were given hydrocortisone (100 mg/kg of body weight) at zero time; they were killed 4 hours later and the enzyme activity in their livers was determined. Radiation from a $Co^{60}$ source was given in the indicated total dosage over a period of 1 hour, beginning 10 minutes after the administration of hydrocortisone. See text for further details. HC, hydrocortisone.

| Time animals were killed (hr after radiation) | Enzyme units (micromoles per hour per gram of liver) |
|---|---|
| 0 Time (control) (6) | 20.5 ± 7.4 |
| 4 hr + HC (5) | 50.2 ± 17.8 |
| 4 hr + HC + 50 r (5) | 89.4 ± 14.9 |
| 4 hr + HC + 300 r (5) | 103.0 ± 14.4 |
| 4 hr + HC + 1600 r (4) | 109.7 ± 17.5 |
| 4 hr + HC + 3200 r (4) | 137.7 ± 39.2 |

The fact that high doses of $\gamma$-radiation did not inhibit the steroid-mediated induction of tyrosine $\alpha$-ketoglutarate transaminase is difficult to understand in the light of our present knowledge. Greengard (*12*) reported the sensitivity of the induction of this enzyme to both actinomycin and puromycin, which indicates a requirement for both mRNA and protein synthesis in the process. Kenney and his coworkers (*10*) demonstrated by the la-

beling and isolation of nuclear RNA that administration of cortisone stimulated precursor incorporation into nuclear RNA. Furthermore, Kenney (*13*) showed that corticosteroid administration resulted in an actual increase in the rate of synthesis of tyrosine transaminase, in complete analogy to enzyme induction in microorganisms. Since this process is so like that in microorganisms where both ultraviolet radiation and x-rays cause an inhibition of enzyme induction, it is not clear why high-energy radiation does not inhibit the steroid induction of this enzyme in mammals. An interesting possibility in view of the resistance of serine dehydrase and ornithine transaminase induction during periods of template stability (actinomycin D resistance) is that tyrosine transaminase is also synthesized on a stable template and that the steroid actually alters the rate of enzyme synthesis at the mRNA stage rather than the stage of DNA-dependent RNA production. The effect of actinomycin in inhibiting corticosteroid induction of tyrosine transaminase may be related to some other action of the antibiotic, such as that reported by Revel and Hiatt (*14*).

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## Evolution of Fitness in Experimental Populations of *Drosophila serrata*

Abstract. *Changes which enhance adaptedness to the environment occur in experimental populations of* Drosophila serrata *which are acted upon by strong natural selection. The improvement is greater in hybrid than in single-strain populations because genetic variability is greater in the former.*

Evolutionary changes can be observed not only on the geological time scale. Rapid adaptive changes have been found in both natural and experimental populations. Among insects, adaptive changes in the genetic constitution of natural populations, caused by natural selection in changing environments, have been recorded in several species (*1*); in some cases these genetic changes have also been reproduced experimentally (*2*). With *Drosophila*, improvements in fitness in carriers of certain chromosomal arrangements have also been observed in the laboratory (*2, 3*). Experiments devised to observe and measure improvements in the ability of a population to exploit the resources of a particular environment are nevertheless rare (*4*). The experiments reported here were designed to study changes of this kind.

Two strains of *Drosophila serrata*, derived from impregnated females collected in nature, were used. One was collected near Popondetta, New Guinea, and had been maintained in the laboratory by mass culture for 1½ years before the beginning of the experiment. The other one was collected in Bulahdelah, about 130 miles north of Sydney, Australia, and had been maintained by mass culture for about 3 years. Six experimental populations were used. Two of the populations were derived from the Popondetta strain and two from the Sydney strain; the other two populations were started with $F_1$ descendents of mass crosses between the Popondetta and the Sydney strains.

One population of each pair was maintained at 25° ± 0.5°C and the other at 19° ± 0.5°C. Each population was started with 150 pairs of flies. The populations were maintained in ½-pint milk bottles, with a ¾-inch layer of medium (cream of wheat and molasses) and with a double piece of toweling, 2 by 7 inches, partially pressed into the medium. The technique has been described in detail elsewhere (*5*). In short, the adult flies are introduced into a bottle with

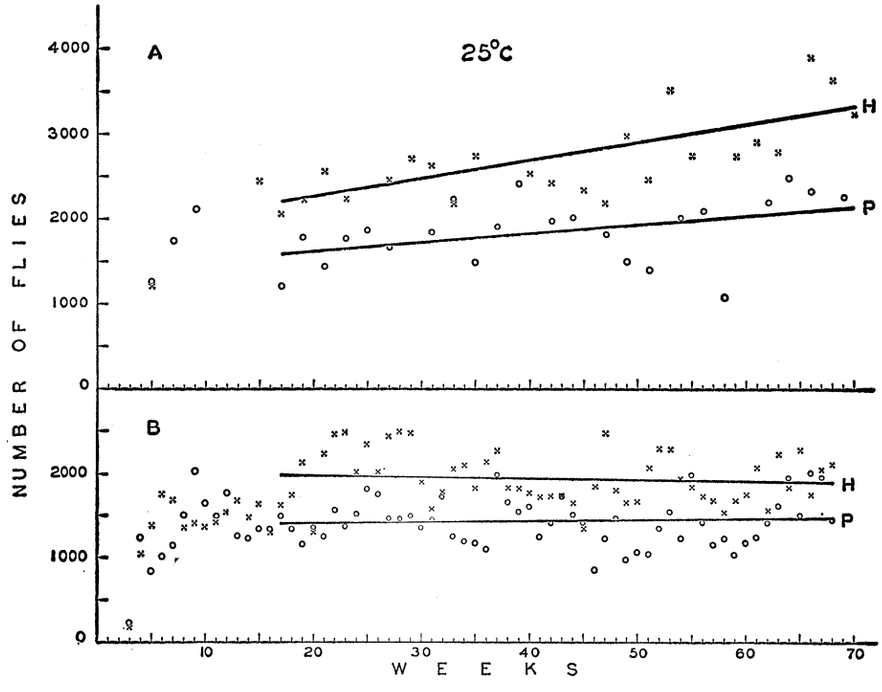


Fig. 1. Population size (*A*) and weekly production (*B*) of two experimental populations of *Drosophila serrata* at 25°C. *H*, Hybrid population; *P*, single-strain (Popondetta) population.