to 19 days. Survival time in populations maintained on the H_2O medium decreased with increasing temperature from 89 days at 20°C to 51 days at 25°C and 25 days at 30°C.

The differential effect of H_2O and D_2O on survival [mean survival time on D_2O medium minus mean survival time on H_2O medium (Fig. 1, inset)] decreased with increasing temperature, becoming very small (6 days) at 30°C.

Deuteration of biological systems has been shown to decrease the rates of essentially all biological processes for which its effect has been studied (*1*). The effect of deuterium has been compared to the effect of decreasing temperature on temperature-sensitive processes; this has been referred to as the "low temperature equivalence" hypothesis (*2*).

If, as has been postulated (*2*), deuteration of biological systems with D_2O decreases the "apparent biological temperature," this mimicry may be expected to extend to survival time in populations of poikilothermic organisms such as *Drosophila*.

It has been suggested that decreasing temperature increases survival time in populations of poikilothermic organisms either through a decrease in the rate of senescence [the "rate of living theory" (*3, 4*)] or through a temperature-dependent change in the "vitality threshold" (*5*). These ideas have received much attention since the early work of Loeb and Northrop (*6*) and Alpatov and Pearl (*4*). Maynard Smith (*5*) has taken exception to the idea that the temperature effect on survival in populations of poikilotherms is due to a decreased rate of senescence at lower temperatures. On the basis of the effect of temperature change during adult life in populations of *Drosophila subobscura*, he proposed that the rate of senescence is unaffected by temperature, but the "vitality threshold" is temperature-dependent.

Strehler (*7*) suggested that decreasing body temperature could result in a "considerable increase in longevity" in homeothermic organisms if a means could be found to compensate for any intolerable temperature-dependent decrease in the rates of physiological processes. Since lowering the body temperature of homeotherms by changing the environmental temperature is not feasible, the desired result may be achieved by chemical mimicry without resorting to manipulation of body temperature.

Our findings place in question the notion of deuterium mimicry of temperature effects on biological systems as well as the notion that temperature affects survival in populations of poikilotherms in a way analogous to its effect on chemical reactions.

The fact that the survival time of *Drosophila* maintained on D_2O decreases with decreasing temperature suggests that effects of deuteration on survival may be due to a binding phenomenon, decreasing the activity of hydrogen at sites of deuteration. Changes in the structure of water due to deuteration may decrease cellular, molecular, and ionic activities to a level which leads to an increase in the probability of death in the deuterated population.

It is possible that the decrement in survival time at any one temperature for flies supplied with medium prepared with 50 percent D_2O may be due to a toxic effect of D_2O at high concentrations which obscures the primary temperature mimicking effect. However, Pittendrigh *et al.* (*2*) demonstrated temperature mimicry of deuterium with 100 percent D_2O.

In our judgment, our findings demonstrate that temperature effects on survival in populations of *D. melanogaster* are not due simply to changes in the rates of temperature-sensitive biological processes of the classical sort, since the survival time of flies maintained on deuterated medium increases with increasing temperature.

Remaining open is the question of whether the deuterium effects observed by us as well as by others (*1, 2, 8*) result from modifications in the properties of the aqueous solvent or from increased deuteration of organic biomolecules.

The effects of deuterium on circadian oscillations (*2*) and the suggestion (*9*) that changes in the temporal organization of biological process may be a signal factor in senescent deterioration indicate to us that differential effects of deuterium on linked biological oscillations may be at the root of the seemingly paradoxical biological effects of deuterium.

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Reduction of Ferricytochrome c by Some Free Radical Agents

Abstract. *Fast pulse radiolysis and kinetic spectroscopy were used to rapidly generate a variety of free radicals in situ and study their reactions with ferricytochrome c in the time range 10^{-6} to 1 second. The radicals included* t-*butanol, which is inert to ferricytochrome c; malate, lactate, and ethanol, which react with it relatively slowly but are completely utilized in reducing it to ferrocytochrome c; and hydrated electrons and hydrogen atoms, which react with it very rapidly but yield ferrocytochrome c only in part, showing intramolecular consecutive reactions and further attack on the ferrocytochrome c protein. From a detailed comparison between malate and hydrogen atoms it is argued that malate reacts directly and selectively with a specific part of the ferricytochrome c surface while hydrogen atoms react with other parts of the protein too, yielding radicals which in part transfer intramolecularly to yield ferrocytochrome c.*

In the structure of ferricytochrome c (CIII) the protein wraps the ferri-heme (*1*) so that only one edge of the porphyrin is exposed, forming some 3 percent of the total surface area of the enzyme. Reduction of CIII to ferrocytochrome c (CII) might proceed by direct reaction of the reducing agent with this defined region or its immediate surroundings, or by reaction with a site or sites elsewhere on the protein followed by intramolecular electron equivalent transfer to the iron moiety (*2*).

The reduction of CIII to CII by

simple one electron equivalent agents, H atoms, hydrated electrons (e^-_{aq}), or organic free radicals under steady state conditions showed (*3–6*) specific differences between yields and products, related, it appeared, to the specific reactivity of the reducing species. To gain information on the details of kinetics and mechanism we have now used the technique of fast pulse radiolysis and kinetic spectroscopy to study the reactions of the organic free radicals derived from butanol, malate, lactate, or ethanol (*4*) with CIII. We compare these with reduction by H atoms (*7*) or e^-_{aq} (*8, 9*) and speculate concerning the role of the protein moiety in the reduction process.

Radiolysis of aqueous solutions is an effective means of producing specific organic radicals which then react with appropriate substrates, for example by reducing them (*10*). By adjusting the relative concentrations, the primary aqueous radicals (e^-_{aq}, H, and OH) may be converted to organic radicals (*11*).

The radiation does not affect the solute CIII; it is merely a convenient source of radicals rapidly formed in situ. The radicals themselves may range from entirely nonreactive with CIII to very reactive.

We used oxygen-free 0.1*M* solutions of malate, *p*H approximately 6.4, saturated with $2.5 \times 10^{-2}M$ N_2O. In the presence of (1 to 6) $\times 10^{-5}M$ ferricytochrome c (Sigma type VI) N_2O converts e^-_{aq} into OH radicals.

$$N_2O + e^-_{aq} \rightarrow N_2 + OH^- + \dot{O}H; \quad (1)$$
$$k = 6 \times 10^9 M^{-1}\ \text{sec}^{-1}$$

These, as well as H atoms, are converted by malate ($RH_2 = {}^-OOC\text{-}CH_2\text{-}CH_2\text{-}CHOH\text{-}COOH$) to the malate radical, $\dot{R}H$

$$\dot{O}H + RH_2 \rightarrow H_2O + \dot{R}H; \quad (2)$$
$$k = (8.6 \pm 0.8 \times 10^8 M^{-1}\ \text{sec}^{-1}$$

$$\dot{H} + RH_2 \rightarrow H_2 + RH; \quad (3)$$
$$k = (5.5 \pm 0.5) \times 10^7 M^{-1}\ \text{sec}^{-1}$$

We calculated the rate constants of reactions 2 and 3 for lactate (7×10^8 and $5.5 \times 10^7 M^{-1}$ sec^{-1}, respectively) from pulse radiolysis experiments which we plan to report in detail elsewhere (*12*).

The malate radicals reduce CIII

$$CIII + \dot{R}H \rightarrow CII + R + H^+ \quad (4)$$

We determined the rate constant for reaction 4 at several wavelengths in the cytochrome c spectrum, concentrations of CIII ranging from (1 to 6) $\times 10^{-5}M$, and doses of 400 to 1500 rads per pulse; the reaction was strictly second order with $k = (8.5 \pm 0.8) \times 10^7 M^{-1}$ sec^{-1}. There was no evidence for any consecutive intramolecular processes within cytochrome c (Fig. 1c). The results with lactate [$k = (2.4 \pm 0.2) \times 10^8 M^{-1}$ sec^{-1}] or ethanol [$k = (1.8 \pm 0.2 \times 10^8 M^{-1}$ sec^{-1}] were similar to those for malate. Tertiary butanol radicals (*13*), which are not reactive with substrates, showed no evidence of reaction with CIII, and protected it fully from the reactions of the primary aqueous radicals.

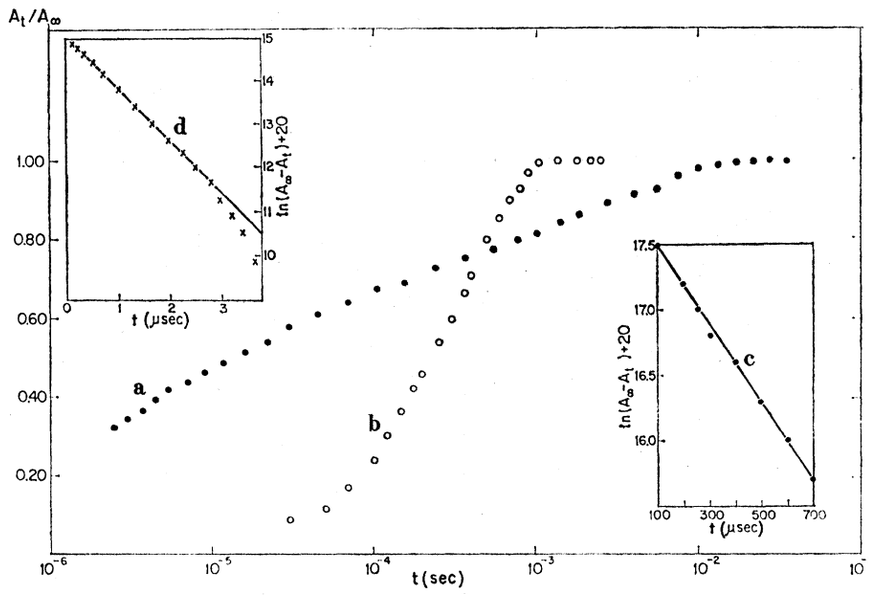

Fig. 1. Reduction of $4 \times 10^{-5}M$ ferricytochrome c to ferrocytochrome c, measured at 550 nm, *p*H = 6.7. The ratio of the absorbancy at time *t* (A_t) to the absorbancy on completion (A_∞) is plotted against log *t*. The absorbancy on completion is taken at 300 msec after the pulse. The reducing species are (a) H atoms and (b) malate radicals. (Inset c) Second order plot for the entire reaction of malate. (Inset d) Second order portion of the initial stages of the H atom reaction; the data are from the reaction at *p*H 3 [the lower *p*H was necessary to increase H atom formation and enable us to use a brief pulse (*7*)].

The results with malate were obtained with the first pulse in a fresh solution. On continued pulsing CIII was quantitatively reduced to CII, and further pulsing did not produce observable spectroscopic changes in CII. From the dose we calculated the yield of radicals and hence the reduction yield from spectral changes at five wavelengths between 375 and 550 nm. The reduction yield is nearly 100 percent. For malate the energy of activation, E_a, was found to be 3 kcal/mole.

In the absence of CIII, malate radicals disappear in a bimolecular reaction, $2k = (2.7 \pm 0.4) \times 10^7 M^{-1}$ sec^{-1}. With various concentrations of CIII and different doses, we calculated the fraction of malate radicals which reacted with CIII. The results showed that all the malate radicals which reacted yielded CII. Similar results were obtained for lactate and for ethanol radicals, which recombine much faster (*14*), $2k = 2.3 \times 10^9 M^{-1}$ sec^{-1}.

These results complement those obtained with steady state radiolysis, which showed that organic radicals have high reduction efficiencies and yield an enzymatically fully active, nonautoxidizable product (*4*).

It is instructive to compare these results with those obtained with e^-_{aq} (*8, 9*) and particularly H atoms (*7*) as the reducing agents. The disappearance of e^-_{aq} in reaction with CIII can be followed; the rate is very fast ($k = 6 \times 10^{10} M^{-1}$ sec^{-1}) and the reduction efficiency is about 75 percent (*9*). The results are consistent with the assumption that at least part of the e^-_{aq} reacts with the protein moiety, some of the protein radicals thus formed transferring intramolecularly to form CII. However, in the case of e^-_{aq}, which reacts with CIII very fast and with the amino acid components of CIII protein relatively slowly (*15*), it could be argued that e^-_{aq} reacts directly with the heme region and that the intramolecular changes observed are mainly conforma-

tional changes involving only minor electron relocalization. This is not so with H atoms as the reducing agent (*7*, *16*). Hydrogen atoms react faster than e^-_{aq} with some amino acid components of protein—for example, aromatic amino acids—but e^-_{aq} react faster than H atoms with CIII. The competition by the protein moiety should be more effective in the case of H atoms. Competition experiments involving steady state irradiation (*5*) or H atoms as such introduced into the solution from the gas phase (*6*) showed that under our conditions all H atoms react with CIII, $k = 1 \times 10M^{-1}$ sec^{-1}. This rate constant was also obtained in the pulse radiolysis experiments, where 15 to 20 percent of the H atoms reacted with CIII in a very fast second order process, yielding CII (*7*). An additional 35 to 40 percent of CII is then formed in intramolecular reactions over the time scale of 10^{-5} to 10^{-1} second (Fig. 1). The total reduction efficiency by H atoms is thus some 50 percent of all H atoms taken up by CIII. Figure 1 shows the kinetics of CII formation with malate and with H atoms. When all the CIII has been reduced to CII by H atoms, the H atoms continue to react with CII, leading—as the steady state chemical experiments showed (*6*)—to reactions with the protein and heme.

Our results thus indicate specific complete reduction of CIII by malate in a second order reaction which is about 30 times slower than the diffusion controlled rate, has a low activation energy, and is thus consistent with selective interaction with part of the enzyme surface. Hydrogen atoms react with CIII at close to the diffusion controlled rate, but only part of H so taken up leads directly to the appearance of CII. The results are consistent with uptake of H by several sites on the enzyme; intermediate enzyme radicals are formed, and the added electron equivalent not residing on the iron part of these radicals transfers intramolecularly to yield CII.

Fast, consecutive intramolecular radical transfer processes have been established in the ribonuclease molecule, and spectroscopic evidence indicated that the sites of the intermediate radicals include divalent sulfur and aromatic amino acids (*17*). For cytochrome c we do not yet know what the sites are.

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Protein Kinase Translocation as an Early Event in the Hormonal Control of Uterine Contraction

Abstract. *β-Adrenergic stimulation with isoproterenol inhibits contractility, increases cyclic adenosine monophosphate (AMP) concentration, decreases the concentration of unsaturated cyclic AMP receptor sites, and increases cyclic AMP–independent kinase in the uterus of ovariectomized rats. The total soluble kinase activity is reduced. The protein kinase activity lost from the cytosol was translocated to the microsomal fraction mostly in a cyclic AMP–independent form, suggesting a particulate substrate for the activated enzyme.*

Regulation of protein kinase activity by hormones has been reported for diaphragm (*1*), adrenal (*2*), and adipose tissue (*3*), but the substrate for activated kinase was not studied; however, the phosphorylase-glycogen synthetase (*4*) and triglyceride lipase (*5*) systems may be assumed to have been involved. Our studies of hormonal regulation of adenylate cyclase in the uterus have led us to investigate the relation of elevation of cyclic adenosine monophos-

Table 1. Effects of isoproterenol on the adenylate cyclase responsive system of rat uterus. Each value is the mean of triplicate determinations and indicates nanomoles of ^{32}P incorporated per milligram of protein.

Protein kinase activity (nmole ^{32}P/mg protein)				Cyclic AMP binding sites (pmole/mg protein)		Cyclic AMP (pmole/mg protein)	
Control		Treated					
−Cyclic AMP	+Cyclic AMP	−Cyclic AMP	+Cyclic AMP	Control	Treated	Control	Treated
10^{-5}M Isoproterenol, 10 minutes							
0.31	2.66	0.86	1.45	3.5	1.7	30.9	490
0.25	1.91	0.41	0.65	2.7	1.0	18	70
0.11	0.51	0.32	0.44	3.6	0.9	9	250
10^{-5}M Isoproterenol, 30 minutes							
0.44	1.19	0.61	0.77	11.4	3.4	3.5	40
0.31	1.48	0.50	0.51	8.0	6.7	6.4	34
5×10^{-6}M Isoproterenol, 20 minutes							
0.24	1.31	0.28	0.81	14.5	11.9	5.4	15
5×10^{-6}M Isoproterenol, 30 minutes							
0.30	1.30	0.50	1.14	12.6	10.2	1.3	1.7
0.35	0.77	0.66	0.77	20.0	12.8	9.4	28
0.28	0.69	0.36	0.41	5.4	4.4	5.7	22