Table 1. Absorption of Fe^{59} in rats maintained on iron-deficient and normal diets.

Diet	No. of days*	Absorbed (% ± S.D.)	No. of rats	P†
Iron-deficient	1	21.9 ± 5.5	10	
Normal		19.9 ± 8.6	10	>.1
Iron-deficient	2	24.0 ± 11.5	11	
Normal		18.0 ± 7.6	11	>.1
Iron-deficient	3	28.6 ± 8.6	10	
Normal		24.5 ± 6.1	10	>.1
Iron-deficient	4	38.7 ± 10.1	12	
Normal		18.7 ± 6.1	10	<.001
Iron-deficient	13	59.3 ± 23.4	11	
Normal		12.7 ± 4.4	11	<.001

* The number of days on which the rats were fed the diet. The number of days of iron deprivation is one more than the number indicated, as the animals were fasted for 24 hours prior to testing the absorption of iron with Fe^{59}. † Statistical comparison is made by using the two-tailed "*t*" test.

absorption in rats maintained on iron-deficient diets for short periods of time. The diet, except for the absence of iron, is nutritionally adequate and consists of casein, sucrose, added vitamins, fat, and minerals. The iron content is 3.9 μg per gram of diet, which is approximately 2 percent of the iron content of the rat diet normally used in our laboratory (*3*).

Systematic study of iron absorption in rats on an iron-deficient diet showed that there was no change in the amount of iron absorbed for the first 4 days of iron deprivation, that there was an increased iron absorption on the 5th day, and that the amount absorbed was markedly increased by 14 days after beginning the iron-deficient diet (Table 1).

The rat loses approximately 0.23 percent of its total body iron per day, and total body iron is approximately 4.2 mg per 100 g of body weight (*4*). At this rate the total amount of iron lost during a 7-day period of iron deprivation, assuming zero iron absorption, would be no greater than 0.15 mg in a 219-g rat. Blood containing this amount of hemoglobin iron was removed from each of a group of rats (mean weight 219 g) by bleeding from the orbital plexus, after which the rats were maintained on a standard diet containing iron. Iron absorption was tested 6 days after bleeding, but no increase in the amount absorbed was demonstrated. Nine control rats absorbed 16.3 ± 4.6 percent of the given dose. Eleven experimental rats absorbed 15.4 ± 5.7 percent of the given dose.

Table 2. The half-life ($T_{½}$) of Fe^{59} in the plasma of rats on iron-deficient diets.

Control		Experimental	
$T_{½}$ (min)	Plasma iron (μg/100 ml)	$T_{½}$ (min)	Plasma iron (μg/100 ml)
66		70	135
56		70	173
66		63	126
66		56	
75	143	69	
68	135	70	
66	168	89	248
72	139	89	176
		75	
		73	190
Mean*			
67		72	

* A *t*-test comparison of the two groups shows no significant difference between the two means ($p > .1$).

The rate of removal of Fe^{59} from the plasma was studied in another group of rats after 7 days of iron deprivation (including 1 day of fasting). The rate in the rats on the iron-deficient diet was similar to the rate in the rats on the normal diet (Table 2).

We considered the possibility that some attribute of the diet, other than the lack of iron, might have caused the increase of iron absorption. Iron was added to the experimental diet in an amount sufficient to equal the iron content of the standard laboratory rat diet (*3*). A comparison of iron absorption in rats maintained on this mixture for 1 week with rats maintained on a normal diet showed no difference. Twelve control rats absorbed 18.9 ± 7.9 percent of the given dose. Twelve experimental rats absorbed 17.8 ± 6.1 percent of the given dose.

An iron-deficient diet appears to increase iron absorption without appreciably changing the total amount of iron stored in the body, as judged by the effect of an equivalent change produced by phlebotomy, and without stimulating erythropoiesis, as judged by the half-life ($T_{½}$) of Fe^{59} injected into the plasma. The possibility exists that an iron-deficient diet may result in prompt depletion of iron in the intestinal mucosa, and that this may be responsible for the increase in iron absorption (*5*). However, the 5-day interval required for iron deprivation to have any effect in increasing iron absorption suggests that the mechanism is more complicated than a direct interaction between luminal iron and the absorptive epithelium because the total population of cells in the mucosal epithelium is replaced in 1.6 days (*6*).

It appears that a partial block to the absorption of a small amount of iron is effected by the iron content of the normal diet. The existence of the block is best appreciated by removing iron from the diet. Since large doses of iron do not appreciably further inhibit iron absorption (*7*), the degree of inhibition would appear to be already near the maximum with a normal diet. The iron content of the rat diet is high as judged by human dietary standards, so that the applicability of these observations to iron absorption in human subjects remains to be proven.

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21 January 1964

Rat Electroretinogram: Evidence for Separate Processes Governing b-Wave Latency and Amplitude

Abstract. *Studies of the simultaneous changes in the latency and amplitude of the b-wave of the rat electroretinogram (ERG) under three different conditions of adaptation show that the latency is primarily a function of the absolute stimulus intensity, being only slightly affected by conditions that strongly reduce the amplitude. This implies that the latency and the amplitude are determined by two independent processes, with the latency-process more closely linked to the initial photochemical events. Furthermore, the different adaptation conditions have surprisingly similar effects on the shape and amplitude of the electroretinogram, which suggests that they all produce only one type of variation in the amplitude-determining process.*

It is well known that in the dark-adapted eye, the latency and amplitude of the b-wave of the electroretinogram (ERG) vary oppositely. When the stimulus-flash intensity is raised, the amplitude increases but the latency

decreases (Fig. 1*A*). It is not so widely appreciated that these features of the b-wave may be varied to some degree independently of each other by varying the conditions of adaptation as well as the intensity of the stimulus. Background light or recent exposure to light greatly reduces the amplitude (*1*), but published curves showing the effect of light-adaptation on the human ERG (*2*) indicate that instead of increasing, the latency in this case seems to stay almost constant or to decrease slightly.

These results suggest that the b-wave latency might be independent of the b-wave amplitude. Because such a functional independence could have important consequences for any theory of the origin and early stages of the ERG, it seemed desirable to study the variation of these two features simultaneously and quantitatively under well-controlled stimulus and adaptation conditions. We chose to work with the albino rat, which has a uniform and essentially all-rod eye (*3*). The electroretinograms were obtained with very short stimulus flashes and with uniform illumination of the entire retina. By this procedure the superposition of inhomogeneous responses was avoided [since the ERG from a corneal electrode is a summation of activity over the entire retina (*4*)]. Thus, this procedure minimized troublesome stray-light responses that occur when only part of the retina is directly illuminated, and also the ambiguities that may arise with long flash durations. Furthermore, it was possible to calibrate stimulus intensities quantitatively in terms of the number of quanta absorbed by the average rod so that the ERG responses could be related to the absolute number of initial photochemical events.

Albino (Sprague-Dawley) rats were anesthetized with Nembutal (50 mg/kg) and maintained at a level of weak tail-reflex. One electrode was placed on the cornea and the other in a small cut in the cheek. Light from a zirconium arc was passed through an interference filter with peak transmission near 500 mμ to eliminate any small contribution of a red-sensitive (possibly cone) system which is present in the rat eye (*5*). An electromechanical shutter was constructed to provide stimulus durations as short as 1.5 msec with opening times of less than 0.25 msec. Uniform illumination of the entire retina was obtained by focusing a large image of the arc so as to uniformly illuminate a section of a ping-pong ball placed over the eye. An absolute calibration of the stimulus intensity, I, in terms of the number of quanta absorbed by the average rod per flash was carried out on some rats by a method reported elsewhere (*6*). The absolute accuracy of the intensity scale for the data given here is estimated to be about $\pm$ 0.4 log unit. The relative intensity of the stimulus beam was controlled to an accuracy of $\pm$ 0.05 log unit.

Three different adaptation conditions were used. (i) Rapid dark-adaptation, in which a moderate adapting flash was presented a fraction of a second before the stimulus flash. This is sometimes called "neural dark-adaptation" (*7*). (ii) Light-adaptation, in which a steady background light was present. (iii) Slow dark-adaptation, in which the eye was exposed for 1 minute to an intense light that bleached a major fraction of the pigment. In this case, the course of dark-adaptation lasted for more than 2 hours after the exposure, the time needed for the visual pigment to regenerate (*7*).

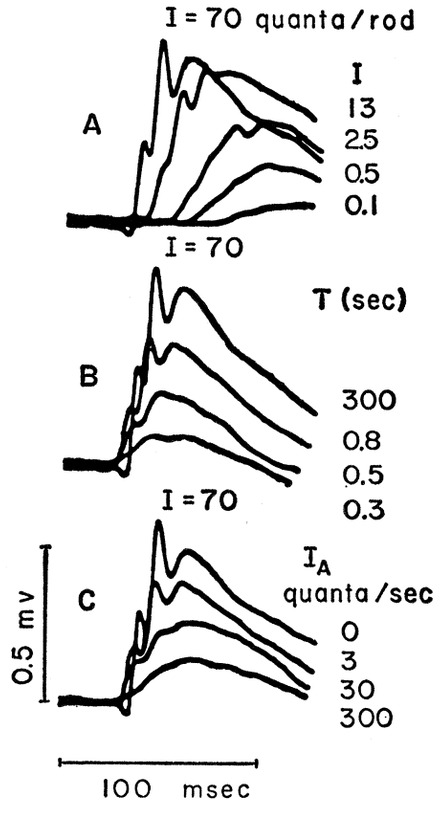

Fig. 1. Electroretinogram of the rat. (*A*) Oscilloscope traces from single stimulus flashes (1.5 msec) with a dark-adapted rat. Numbers beside the traces indicate stimulus intensity, I, in quanta absorbed per flash by the average rod. (*B*) Traces from single stimulus flashes during rapid dark-adaptation after an adapting flash; same rat. Stimulus flash intensity, 70 quanta/rod; adapting flash intensity, 80 quanta/rod. Numbers indicate time, T, in seconds, from adapting flash to stimulus flash. (*C*) Traces from single stimulus flashes in presence of background light; same rat. Stimulus intensity, 70 quanta/rod; numbers indicate background intensity, I_A, in quanta absorbed per second by the average rod.

Typical results are presented in Figs. 1 and 2. Parts *B* and *C* of Fig. 1 show superimposed oscilloscope traces of the ERG under the first two adaptation conditions, and, for contrast, Fig. 1*A* shows how, in the dark-adapted rat, the b-wave amplitude increases and the latency decreases when the stimulus intensity is raised. The b-wave is the prominent wave, with the small negative a-wave appearing only at high intensities. Because the a-wave (Granit's PIII) (*1*) opposes the b-wave (Granit's PII) it is not possible to measure accurately the true latency and amplitude of the b-wave in the normal eye. However, in our study, we wished only to observe changes in the b-wave which were produced by changes in the adaptation conditions. For this purpose, it was sufficient to measure the amplitude of the b-wave from the baseline to the point of maximum excursion, and to measure the latency from the start of the oscilloscope trace, (which was triggered by the flash), to the point where the b-wave rose sharply up from the baseline or from the a-wave (see inserts in Fig. 2).

In Figs. 1*B* and 1*C*, the intensity was set at the highest intensity used in Fig. 1*A*, and then the level of adaptation was varied. In Fig. 1*B*, it can be seen that an adapting flash [condition (i)] strongly reduces the amplitude, but leaves the latency almost unchanged. Figure 1*C* shows that a steady background light [condition (ii)] has essentially the same effects. Rather than an increase in latency with decreasing amplitude, there is, if anything, a small decrease in both cases, from about 34 to about 29 msec. This confirms the result for the human ERG in reference (*2*). In Figs. 1*B* and 1*C*, these decreases in the latency might appear to be artifacts resulting from the disappearance of the a-wave, but they are somewhat larger at lower stimulus intensities where the a-wave is not seen at all. The decrease in latency which occurs when a background light is

present is quite stable. It remains unaltered for at least 40 minutes and is therefore not due to a transient adaptation effect.

Except for these small decreases, for both of these adaptation conditions the latency is primarily determined (to within a few milliseconds) by the stimulus intensity, rather than by the state of adaptation or factors that determine the amplitude. This has been found to be true over wide ranges of stimulus- and adapting-light intensities.

The results obtained with adaptation condition (iii) also point to the same conclusion. This is shown in the comprehensive plots in Fig. 2 which give the b-wave latency, L, and amplitude, A, as functions of the stimulus intensity, first for the dark-adapted eye (solid line for L and top curve for A in Fig. 2); and then at various times over a 2-hour period during the slow recovery of the eye from the bleaching produced by an exposure to intense light. In the example shown, both the rise in the b-wave threshold (*7*) and the known intensity of the bleaching light indicated that about three-quarters of the pigment was bleached away. The stimulus intensities used during the recovery were low enough to avoid retarding the slow regeneration of the pigment.

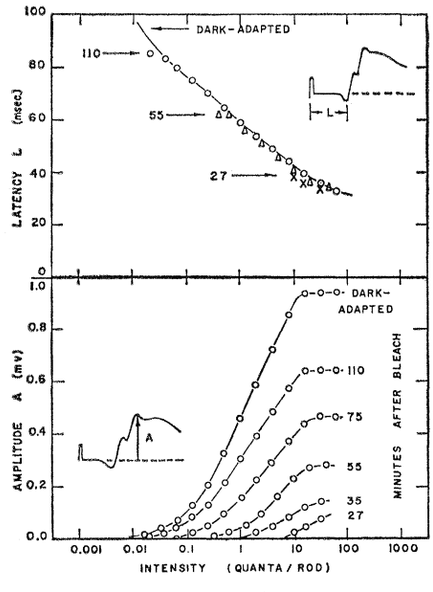

Fig. 2. Electroretinogram of the rat: b-wave latency, L, and amplitude, A, as functions of stimulus intensity, during dark-adaptation after exposure to intense light. Solid line for L and top curve for A refer to the fully dark-adapted eye before bleaching. Heads of arrows indicate threshold at time after bleach: triangles, 110 minutes; circles, 55 minutes; squares, 27 minutes. Logarithmic intensity scale gives the number of quanta absorbed by the average rod per stimulus flash. Flash duration, 10 msec. (Correction for the loss of pigment after the bleach, used to convert incident intensity to absorbed intensity: $\Delta \text{ Log } I = -0.5$ at 27 minutes; -0.3 at 35 minutes; -0.15 at 55 minutes.)

Figure 2 shows that although the amplitude drops by more than a factor of 10 between the extreme curves, the latency of the b-wave for a given stimulus is almost unaffected, so that we can say even more accurately than in the previous cases that the latency is a function of the stimulus intensity alone (*8*). In particular, there is almost no shift in the latency at low intensities, so that the latency is not a function of the state of adaptation even in the region where only an occasional rod absorbs a quantum in each flash. Results similar to those in Fig. 2 have also been obtained with four other rats.

This experimental evidence leads to several conclusions. First, the fact that we can vary the amplitude of the b-wave without much affecting the latency strongly suggests that these two features of the ERG are governed by two independent processes.

More explicitly, the fact that the latency depends so closely on the stimulus intensity alone, suggests that the latency-determining process, whatever it is, must be closely connected to the initial photochemical events in the rods. In particular, it would appear that the physico-chemical processes responsible for the latent period must be relatively unaffected by the adaptation-disturbances that were introduced, namely, recent retinal activity, or concurrent background light, or the presence of large amounts of bleached pigment (see *9*).

It has been generally supposed that the b-wave of the ERG is produced in the bipolar cell layer (*6*, *10*) and that the b-wave latency is related to the time for excitation to reach the ganglion cells after a flash (*11*). Previous experiments on "summation" by the sources of the b-wave have shown that at least part of the latent period must be taken up by processes in the bipolar cell layer (*6*). However, it is hard to see how the present results could be explained without supposing that most of the latent period is determined nearer the photochemical events in the rods.

Finally, the data indicate an important characteristic of the process that governs the amplitude. It can be seen in Fig. 1 that the family of ERG curves for rapid dark-adaptation, Fig. 1*B*, can almost be superimposed on the family of curves obtained on background light, Fig. 1*C*, on the same animal. The family of curves produced during slow dark-adaptation is also similar, although it was not convenient to obtain superimposed oscilloscope pictures in this case. Such a similarity of the ERG curves for the three adaptation conditions has been found in every animal studied. And when the amplitudes of the curves are then plotted for the whole range of stimulus intensity, as in the lower diagram in Fig. 2, these diagrams, for all adaptation conditions, can again be almost superimposed.

Thus, the ERG curves produced under various adaptation conditions appear to form a single, one-parameter family of curves for each stimulus intensity, with almost the same curve shape generated for a given amplitude regardless of which adaptation condition has held down the generating process to this amplitude. Therefore, all three adaptation conditions appear to affect the amplitude-governing process equivalently because all three appear to produce variations in only a single parameter of the process. This equivalence is an unexpected simplicity in the workings of the retina when one considers the great differences between the three adaptation conditions and the possible multiplicity and complexity of the sources of ERG.

While we do not yet know what these characteristics of the b-wave may mean in physical and chemical terms at the cellular level, it is clear that the functional independence of the latency and the amplitude of the b-wave must be taken into account in any theory of the origin and early stages of the ERG. A more complete account of the experiments will be published elsewhere (*12*) together with an account of further experiments on the additivity properties of the latency-determining process.

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9. It is interesting to compare these results on the rat ERG with those found by Hartline for spikes from a single optic nerve fiber in *Limulus*. The latency of the first spike is little changed during slow dark-adaptation, but the number and frequency of spikes is greatly altered, like the ERG amplitude here. [H. K. Hartline, *J. Opt. Soc. Am.* **30**, 239 (1940), Figs. 5 and 6.]
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Actinomycin D and the Response to Parathyroid Hormone

Abstract. *Actinomycin D inhibits the effect of parathyroid hormone upon bone, as measured by calcium mobilization, without altering its effects upon the renal excretion of phosphate and calcium.*

The administration of parathyroid hormone to a parathyroidectomized animal produces a characteristic sequence of events. An immediate increase (within 10 to 20 minutes) in the renal excretion of phosphate, a decrease in calcium excretion, and a concomitant fall in the concentration of phosphate in the plasma are followed by a gradual rise in the concentration of calcium in the plasma (*1*). The rise in plasma calcium may not appear for an hour or more and it is eventually accompanied by a progressive increase in the excretion of calcium in the urine. Once established, hypercalcemia and hypercalciuria persist and their magnitude are proportional to the dose of administered hormone. This change in plasma calcium is thought to result from an increased absorption of calcium from the gastrointestinal tract, an increased reabsorption of calcium by the renal tubule, and the mobilization of calcium from bone (*2*, *3*). The last is by far the most important in quantitative terms. The response of the gastrointestinal tract appears to be least important. Thus the two major organs which respond to this hormone are kidney and bone. There is good evidence that the hormone acts directly on both organs (*3*). The renal response is characterized by a rapid onset which is limited in magnitude, and a high sensitivity to small changes in hormone concentration. The response of bone on the other hand is sluggish in onset, relatively insensitive, but of a nearly unlimited capacity. The basis for this difference in the time course and sensitivity of their responses is not known. We suggested previously that the difference may be due to the striking difference in the rate of blood flow to these two organs (*2*). Further consideration has led to an alternative or perhaps complementary explanation for these differences.

Parathyroid hormone exerts a direct stimulatory effect upon the process of bone resorption (*4*); the process is highly complex and poorly understood, but current opinion favors the view that resorption of bone is brought about by specialized cells, osteoclasts (*3–7*). Furthermore, all available histological evidence indicates that these cells have arisen from other types of bone cells, and that they have an average lifetime of 36 to 48 hours (*6*). In view of these facts, a fundamental action of parathyroid hormone upon bone cells may be that of initiating the development or differentiation of bone-destroying osteoclasts. In such a case, the time course of hormone response in this organ might well be a reflection of the rate of cellular differentiation.

It could be predicted that underlying this hormonally induced change in cell structure and function would be a change in the rate of synthesis of either the type or the amount, or both, of ribonucleic acids and proteins within these cells. If this were the case, then the administration of agents known to block these synthetic activities should lead to an inhibition of hormone action upon bone.

Of the many agents which have been reported to interfere with either RNA or protein synthesis, actinomycin D appears to be one of the most specific and best characterized (*8*). It appears to block the DNA-directed RNA synthesis catalyzed by RNA polymerase by binding at specific sites on the DNA primer.

In order to test our hypothesis, we followed the time course of the change in the concentrations of plasma calcium and phosphate after the administration of purified bovine hormone (*9*) to either parathyroidectomized animals or parathyroidectomized animals which had received 1 μg of actinomycin D per gram of body weight 2 hours before administering the hormone. Parathyroidectomy was performed several

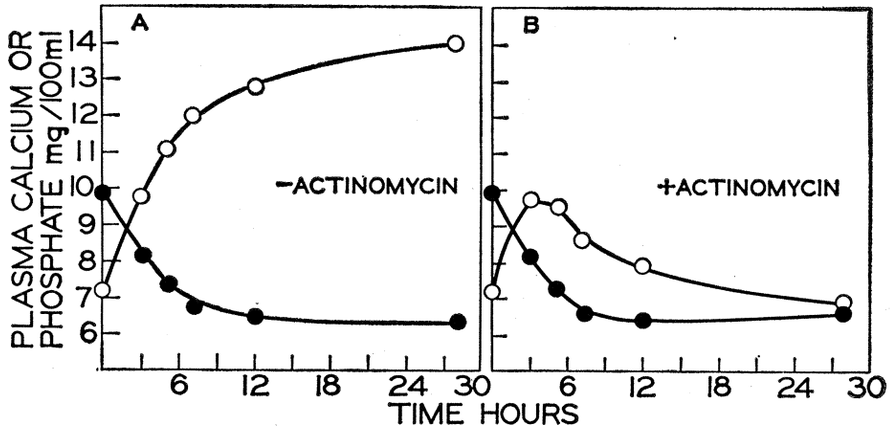

Fig. 1. The changes in plasma calcium (open circles) and plasma phosphate (closed circles) as a function of time after the intraperitoneal injection of 200 μg of purified bovine parathyroid hormone into parathyroidectomized rats weighing 150 g. The values on the left are for control animals, and those on the right for animals given 1 μg of actinomycin D per gram of body weight 2 hours before the injection of hormone. Each point represents the mean of the plasma values of 12 rats.