into the bone marrow cavity contain < 10 parts per billion (ppb) uranium, while the bone itself, to which crystals are attached, contains 2.3 parts per million (ppm). The uranium concentration in the bulk of the breccia away from bone fragments is ~ 80 ppb. While bone is well known to be an open system with respect to uranium [for example, see (*8*)], the calcite appears not to have accumulated uranium since its crystallization.

The age of Bed I, Olduvai Gorge, is well documented at ~ 2 million years (*9*). For a uranium concentration of 40 ppm (Table 1) the expected track density is $\sim 4 \times 10^4$ tracks per square centimeter. However, no spontaneous tracks were seen in the two calcite samples from Bed I. We also observed no spontaneous tracks in the calcite samples from the South African cave deposits. Hay (*7*) has assured us that the Bed I crystals have never experienced temperatures greater than ~ 30°C. According to Sippel and Glover (*2*) tracks in calcite should be completely unaffected by this low temperature over 2 million years. Thus, it was necessary to redo the heating experiments in order to check the accuracy of the reported annealing characteristics.

Track fading can be described by an equation of the type $t_a = Ae^{U/kT}$, where t_a is the annealing time for a given track density reduction, U is the activation energy, T the temperature, k Boltzmann's constant, and A another constant. Our heating experiments, performed on neutron-irradiated Olduvai Gorge calcites, indicate that the activation energy for complete track erasure is similar to that reported by Sippel and Glover (*2*) (35 kcal/mole or 1.5 ev) but that the temperatures for annealing (over a given time) are considerably lower (that is, the value of A in the annealing equation is different). Our data, extrapolated to 2 million years, indicate that tracks will anneal completely at a temperature of ~ 20°C. The difference between our results and those of Sippel and Glover (*2*) can be attributed to two factors: (i) The etching solution used by them produced pits difficult to distinguish from dislocations. Since dislocations anneal at higher temperatures than do fission tracks, this may partly account for the higher temperatures they report. (ii) They did their annealing measurements on an exterior surface that had been irradiated with fission fragments, whereas our experiments were done with neutron-induced fission tracks crossing an interior surface. It is known (*10*) that tracks crossing external surfaces are more resistant to thermal annealing than are internal tracks. The latter approximate the natural case better than the former.

Table 1. Uranium concentration in the calcite samples studied.

Sample	Uranium content (ppm)
Bed I, Olduvai Gorge	
69-6-17-G	39
62-7-9-U	40
Bed II, Olduvai Gorge	
64-7-7A	44
70-6-29M	0.049
Swartkrans travertine	1–2*
Makapansgat (marrow cavity)	
Pink breccia, lower cave	< 0.010
Grey breccia, lower cave	< 0.010

* Variable.

Our annealing experiments indicate that the lack of spontaneous fission tracks in the calcite samples is due to track annealing at ambient temperatures. We were unable to locate any more thermally resistant phases suitable for fission track dating in the South African samples, and in fact it is unlikely that any exist in a limestone terrain. Thus, disappointingly, it appears that fission track dating will not provide an absolute chronology for the South African Australopithecines and that in general calcite will not be a useful mineral for fission track dating.

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Acetylcholine Noise: Analysis after Chemical Modification of Receptor

Abstract. *The elementary voltage pulses ("shot effects") produced by the action of acetylcholine molecules on the receptor were studied by analyzing the membrane voltage fluctuations ("noise") after acetylcholine application at the frog neuromuscular junction. The amplitude of these pulses was decreased after treatment with a disulfide-bond reducing agent. The shot effect may thus depend on the structure or conformation of the receptor molecule.*

Interaction of transmitter molecules with their specific receptors produces an ionic permeability change in the postsynaptic membrane. This change permits the flow of synaptic current which, in turn, depolarizes the membrane. A new approach to understanding this process was introduced by Katz and Miledi (*1*), who succeeded in studying transmitter-receptor interaction at a molecular level by analyzing fluctuations in membrane voltage after acetylcholine (ACh) application, a phenomenon termed "ACh noise." They showed that the postsynaptic effect of ACh is composed of a great number of elementary events which they called "shot effects." These presumably reflect the activation of single receptor molecules by the transmitter. They also showed that different agonists (such as carbachol) produce different shot effects. To see whether the shot effect could also be modified by causing a structural change in the receptor molecule itself, we treated a cholinergic synapse with dithiothreitol (DTT), which decreases the postsynaptic sensitivity to ACh by reducing a specific disulfide bond situated a few angstroms away from the anionic site of the receptor (*2, 3*). This effect can only be reversed by subsequent reoxidation, for instance, by 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) (*2, 3*). We now report that treatment with DTT affects the elementary permeability events produced by ACh, probably by decreasing both their amplitude and duration.

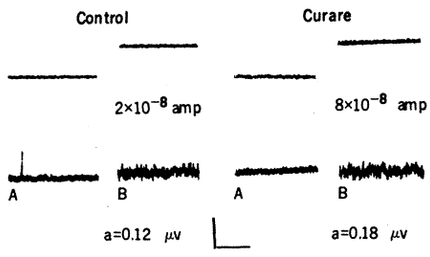

Fig. 1. Acetylcholine noise (A) before iontophoresis of ACh and (B) during iontophoresis. Magnetic tape playback was recorded on chart paper after eightfold reduction of the original tape speed. Upper traces are low-gain d-c recordings of the cell membrane potential. Lower traces are high-gain a-c recordings of the membrane potential. The resting potential of the cell during the experiment was −82 to −79 mv, and application of ACh produced a depolarization of 8 mv. A single miniature end plate potential (MEPP) is seen in the control (A); MEPP's disappeared after treatment with curare (10^{-6} g/ml). The iontophoretic current is indicated between the upper and lower traces. At the bottom, the calculated amplitude of the shot effects (a) during ACh applications is indicated. Calibration: voltage, 10 mv for the upper traces and 250 μv for the lower traces; time, 500 msec.

The experiments were performed on the frog sartorius neuromuscular preparation in vitro at room temperature (20° to 24°C). In some experiments, preparations denervated for 8 to 11 days were employed in order to avoid contribution to the ACh noise from undetected miniature end plate potentials. The bathing medium was composed of 115.0 mM NaCl, 2.0 mM KCl, 0.5 mM $CaCl_2$, 2.5 mM $MgCl_2$, and 1.0 mM tris(hydroxymethyl)aminomethane buffer (pH 8.0). Drugs employed were acetylcholine chloride (obtained from British Drug Houses), d-tubocurarine (curare, from Taro, Haifa, Israel), and DDT and DTNB (both from Sigma). Conventional methods were used for intracellular recordings, focal extracellular recording, stimulation, and iontophoresis of ACh (*4*). For recording ACh noise, both low-gain and condenser-coupled (*5*) high-gain records of the intracellular potential were made on a magnetic tape recorder (Hewlett-Packard 3902 system) and stored for further analysis.

Acetylcholine noise is shown in Fig. 1. The application of ACh to an end plate region produced a postsynaptic depolarization of 8 mv (upper traces in Fig. 1). On the high-gain recording (lower traces in Fig. 1), an increase of the membrane voltage fluctuations (noise) can be seen. This noise, which could be detected only upon positioning of the drug pipette in the end plate region, was often obtained just by reducing braking current and increased with membrane depolarization in the range of our observations (up to 16 mv). No similar noise was observed when the direction of current was reversed or when the ACh pipette penetrated the muscle fiber. Also, applications of ACh repeated after a minimum delay of 10 minutes produced identical noise. In order to determine the variance and power spectral density of the noise, ACh was applied for 30 to 60 seconds, and 1-second sections from the high-gain record were sampled at 2.5-msec intervals, by the use of the analog-to-digital converter of a small computer (CAT 400C, Technical Measurement Corp.) connected to a printer. The data were further analyzed on the Control Data Corp. 6600 digital computer of Tel-Aviv University. Usually, 5 seconds from each application of ACh were thus analyzed. The system was calibrated by sine-wave oscillations of known frequency and amplitude. The amplitude of the shot effect (a) was calculated from the variance of the noise by the equations of Katz and Miledi (*1*) and with the precautions they advised. The average a in 17 different preparations was 0.18 ± 0.02 μv (mean ± standard error), a value smaller than that reported by Katz and Miledi but within the range of their observations. The power spectral density of the noise was computed by employing computer program BMD02T (*6*). As found by Katz and Miledi (*1*), the noise was confined to a rather low frequency spectrum. The time constant of decay of the shot effect, as derived from the power spectrum, was 6.6 ± 0.7 msec ($N = 17$), corresponding to a half-power value of 24 hertz.

Before employing DTT, we tested our system with curare, a drug of known effect which decreases synaptic sensitivity to ACh (as does DTT) but does not modify the shot effect (*1*). The results of an experiment with curare (10^{-6} g/ml, 10 minutes) are shown in Fig. 1. Synaptic sensitivity to ACh was reduced, since four times as much current was necessary to produce the same depolarization as in the control (that is, before curare treatment). However, ACh noise was increased and the calculated shot effect, a, was 63 ± 18 percent greater than the control value in four innervated and two denervated preparations. Such a change after curare treatment was not found by Katz and Miledi (*1*); indeed, when

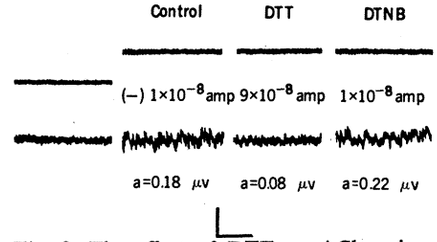

Fig. 2. The effect of DTT on ACh noise. The preparation had been denervated for 8 days. The upper and lower traces are low- and high-gain records of the membrane potential as in Fig. 1. The resting potential of the cell was −84 to −78 mv during the experiment. The ACh potential before treatments (control), in DTT (1 mM, 45 minutes), and in DTNB (1 mM, 75 minutes) was always 8.5 mv. The traces on the left are voltage recordings at the beginning of the experiment, before application of ACh. The background noise was stable during the experiment. The iontophoretic currents are indicated as in Fig. 1. In the control, effective iontophoresis was achieved just by reducing the braking current to 1×10^{-8} amp. This is denoted by a minus sign. The amplitude of the shot effects is also indicated as before. Calibration: voltage, 8.4 mv for the upper traces and 210 μv for the lower traces; time, 500 msec.

these experiments were repeated in the innervated cutaneous pectoris preparation from frogs and positioning of the electrodes was better controlled by the use of Nomarsky optics (*7*), the increase of the shot effect after curare was only 12 ± 9 percent ($N = 10$) of the control value (*8*). We believe that this increase may have been caused by a pipette artifact, such as fluctuations in the resistance of the ACh pipette (*1*) that produced corresponding fluctuations in the drug efflux. This, in turn, would tend to produce an overestimation of the ACh noise, especially when synaptic sensitivity was reduced and larger ACh currents were needed to depolarize the end plate. Such an artifact may largely be avoided when Nomarsky optics are used, because much smaller ACh currents are then required to produce a given effect.

The effect of DTT was then examined. As shown in Fig. 2, the application of DTT (1 mM, 45 minutes) markedly reduced the end plate sensitivity to ACh; in contrast to curare, the ACh noise was also lowered. Both effects could be reversed by DTNB (1 mM, 75 minutes). In 11 experiments like that of Fig. 2, the average reduction of a after treatment with DTT (0.5 to 2.0 mM, 10 to 65 minutes) was to 50 ± 7 percent of the control value (*9*). This result was particularly meaningful in view of our tendency

to overestimate a when synaptic sensitivity to ACh was decreased. Of our experiments, six were in innervated preparations and five were in preparations denervated for 8 to 11 days. The a values in the innervated preparations were 60 ± 10 percent of control values, and those in the denervated preparations were 38 ± 2 percent of controls. We have no explanation for the larger effect in the second group. When DTNB (1 mM) was tested in four of the denervated preparations, it produced a recovery of a to 92 ± 17 percent of the control value (that is, value before DTT) within 25 to 80 minutes. The effects of DTT and DTNB were also confirmed in the innervated cutaneous pectoris preparation (*10*).

The shot effect derived from intracellular voltage measurements offers only a distorted view of the underlying permeability change. Its time constant of decay reflects primarily the surrounding membrane characteristics rather than the relaxation time of the elementary permeability event. Both a diminution in amplitude and shortening of this event would appear as a reduction of a (*1*). In order to decide which of these two mechanisms was responsible for the effect of DTT on a, we resorted to extracellular recording of end plate potentials (EPP's). The time course of decay of these potentials, when measured in the absence of Prostigmine, does not differ greatly from the relaxation time of the elementary permeability change produced by ACh (*1, 11*). Extracellular EPP's were recorded at 12 different junctions, and the effects of DTT (1 mM) were examined at various times between 10 and 65 minutes. In all of these experiments, DTT produced a strong reduction of the EPP amplitude which was fully reversed by DTNB. In the first 30 minutes, the effect of DTT on the duration of the EPP was small, and the decay half-time ($t_{1/2}$) declined on the average to 93 ± 10 percent ($N = 12$) of control values. However, with longer periods of treatment (35 to 65 minutes), a marked shortening of the EPP duration was evident, and the average $t_{1/2}$ declined to 59 ± 9 percent ($N = 7$) of control values. This effect was also fully reversed by DTNB. This result, which indicates an effect of DTT on the duration of the permeability event, may partly account for the observed reduction of a. However, in contrast to the change in $t_{1/2}$, most of the DTT-produced decrease in a had already appeared after 30 minutes of treatment [after this interval, a was 58 ± 9 percent of the control value ($N = 7$)]. It is therefore likely that a decrease in the amplitude of the elementary permeabilty event had also occurred.

In conclusion, it appears that one can obtain a different permeability event not only by modifying the structure of the agonist (*1*), but also by modifying the structure or conformation of the receptor. In our study, the reduction of a specific disulfide bond located near the anionic receptor site (*2, 3*) sufficed to produce a markedly reduced permeability event. This result indicates that a partial inactivation of the basic excitation unit is possible. This would occur if the receptor were comprised of several subunits (*12*) which could be separately affected by DTT treatment.

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10. In two preparations, one treated with DTT for 7 and the other for 15 minutes, a decreased to 69 and 64 percent of control values, respectively. In two other preparations, one tested for 20 minutes and the other for 30 minutes, the respective values of a were 50 and 37 percent of controls. In the last experiment, treatment with DTNB for 60 minutes produced a recovery of a to 95 percent of control values (D. Ben-Haim, F. Dreyer, K. Peper, in preparation.
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Hemoglobin Switching in Sheep and Goats: Occurrence of Hemoglobins A and C in the Same Red Cell

Abstract. *Sheep and goats switch from the synthesis of hemoglobin A ($\alpha_2\beta_2^A$) to hemoglobin C ($\alpha_2\beta_2^C$) when made anemic. We have demonstrated the existence of the asymmetrical hybrid hemoglobin, $\alpha_2\beta^A\beta^C$, in the circulating red cells of anemic sheep. These erythroid cells, therefore, synthesized both A and C hemoglobin simultaneously. Thus, the switch appears to be mediated by selective gene expression rather than by a clonal or cellular selective mechanism.*

Hemoglobin synthesis in goats and certain sheep represents a unique model for the study of regulation of gene expression in eukaryotic cells. The erythroid cells of these animals switch from the synthesis of hemoglobin A ($\alpha_2\beta_2^A$) to hemoglobin C ($\alpha_2\beta_2^C$) in vivo in response to anemia, hypoxia, or erythropoietin injection (*1*) and in vitro in tissue culture in the presence of erythropoietin (*2, 3*). Gabuzda *et al.* (*4*) showed that the switch in β globin synthesis in vivo is detectable in the marrow 3 to 5 days after erythropoietin injection. Barker *et al.* (*3*) have found a similar time course in tissue culture. We interpret these data to mean that expression of the β globin gene (or genes) is determined early in erythroid cell maturation. To explore further this model of gene regulation, we found it desirable to ascertain whether control of globin synthesis is exerted by selective stimulation of cells capable of making only one type of β globin (cell selection), or whether both β chains are synthesized simultaneously in each cell (intracellular regulation).

Our study was facilitated by our knowledge of the fact that asymmetrical hybrids of hemoglobin exist in solution (*5*). Hemoglobin is a tetrameric molecule consisting of two α globin subunits and two β globin subunits. The tetramer readily disassociates into dimers as follows: $\alpha_2\beta_2 \leftrightarrow 2\ \alpha\beta$. In a mixture of hemoglobins, reassociation of unlike dimers gives rise to asymmetrical hybrids. For example, one might expect in a mixture of hemoglobins A ($\alpha_2\beta_2^A$)