cently publicized a variety of products elaborated to a greater or lesser extent and unexpectedly by different strains of HeLa. Briefly, two purported "breast carcinoma" lines, G-11 and HT-39, produce "α"-lactalbumin (*8*); a "prostate adenoma," MA160, shows C_{19}-radiosteroid metabolism of prostatic epithelium (*9*); "lung tumor cells," 2563 (= MAC-21), produce antibodies in rabbits that act specifically against human lung tissue (*10*).

A hypothesis of genetic derepression seems to be the simplest explanation for these results in variants of HeLa cells, derived as they probably were from a single cell. While certain physical characteristics (marker chromosomes) remain virtually unaltered, and many genetically determined gene-enzyme systems remain constant in all HeLa cells, some strains of these aberrant human cells are generating unexpected products similar in a sense to "ectopic" behavior of tumor cells in vivo. Alternatively, one may be detecting fetal antigen activity.

It appears that a single culture of HeLa cells cannot now be considered as the sole representative of this vast family of cell strains in terms of products; a condition which may well be the fate of all bona fide long-term cultivated cell lines regardless of tissue of origin.

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Membrane Potentials of Mitochondria

Maloff *et al.* (*1*) claim that "the membrane potentials of giant mitochondria from cuprizone-fed mice were found to be independent of metabolic state." This conclusion, which is based on the use of microelectrodes, is in sharp contrast to a large body of evidence obtained from various mitochondrial systems by a variety of techniques [see (*2*)]. Maloff *et al.* also claim that "experiments are described in which the presence of the microelectrodes in the inner mitochondrial space, . . . are validated." The only experiment brought as evidence for the latter claim is the response of the microelectrode signal to the addition of valinomycin and NaSCN. The potential which is positive initially is not affected by the addition of succinate but is slowly reversed by the addition of valinomycin and further reversed by the addition of NaSCN [see (*1*, figure 1A)]. Maloff *et al.* argue that the reversal of the polarity is the result of the formation of a diffusion potential across the mitochondrial inner membrane, the inner space of this membrane having internal K^+ and no SCN^- anion. Thus, they argue, valinomycin (which increases membrane permeability to K^+) induced a diffusion potential across the inner membrane, the inner space thus being negative. Hence, the reversal of the electrode polarity indicates its location in the mitochondrial matrix. Similarly, the addition of the lipophilic anion SCN^- would induce diffusion potential across the inner membrane, making the inner space negative. I would like to point out that the microelectrode tip also has a very high potassium concentration (2*M*) and no SCN^- anion. Thus, for instance, if the electrode tip became obstructed by a membrane or hydrophobic material, valinomycin (and SCN^-) would induce the formation of a negative potential. A membrane-coated microelectrode is very much like a K^+ specific membrane-electrode or other ion-specific membrane electrodes. In fact it is not unlikely that the inner membrane of the mitochondria, which has a very large surface to volume ratio and is not a rigid sphere, simply engulfs the electrode tip without being penetrated and thus converts the electrode into an ion-specific membrane electrode. In this event the electrode responds to valinomycin and SCN^- but not to any metabolic event in the mitochondrion. Recent experiments with both intrinsic and extrinsic membrane potential probes (*3*) have indicated that unlike the experiments of Maloff *et al.* (*1*), K^+ diffusion potentials when induced by valinomycin in nonenergized mitochondria are formed within 1 second and decay fairly rapidly with a half-time of 10 to 30 seconds. This decay is due first to the movement of other ions, mostly protons, in exchange for K^+ and second to the consequent slower depletion of the mitochondrial K^+. Moreoever, after an addition of succinate the potential which is generated by the proton pump is negative and high (*4*), and the addition of valinomycin, in the presence of external K^+ (5 m*M*), decreases the high negative value by about 50 mv opposite to what Maloff *et al.* (*1*) observed. This effect is due to the influx of K^+ into the matrix in contrast to the euflux which is generated in nonenergized mitochondria. Thus, it is suggested that Maloff *et al.* do not measure potentials across the inner mitochondrial membrane but probably across the obstructed electrode tip.

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The artifactual formation of a membrane capable of responding to valinomycin at the electrode tip can be ruled out by a variety of arguments:

1) The addition of valinomycin does not change the membrane potential when the mitochondria are in a medium approximately between 60 and 80 m*M* (*1*). This is consistent with an intramitochondrial K^+ concentration in that range and not with the 2*M* KCl contained in the microelectrodes. The alternative, that a pocket is formed at the mitochondrial membrane into which K^+ leaks from the electrode, is also unlikely. The potentials do not change significantly with time (up to 3 minutes) and are independent of the electrode taper and size of the tip (as measured from the electrode resistance) (*2*).

2) The results obtained with microelectrodes correspond quantitatively to those obtained with electrofluorimetric dyes in either *Drosophila* (*3*) or giant mitochondria of mice (*4*).

3) Finally, the same results are obtained whether the electrodes are filled with KCl or NaCl. Figure 1 shows the results of an experiment carried out with an electrode containing 2*M* NaCl. For six impalements the means (± standard deviation) were 16.8 ± 2.6 mv and −15.5 ± 2.8 mv in the absence and in

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the presence of valinomycin, respectively. When the electrode was withdrawn (third arrow) the potential returned to approximately the original value. In a similar experiment (*1*) at the same KCl concentration (1 m*M*) and with electrodes filled with 2*M* KCl the corresponding potentials were 16.8 ± 1.0 and −14.0 ± 3.0 mv.

The experiments discussed by Rottenberg (*5*) and presumed to provide evidence for a metabolically induced membrane potential, are most readily interpretable on the basis of a nonelectrogenic H^+ pump (*6*). In this model the H^+ exchanges for a cation for which the mitochondrial membrane is sufficiently permeable (for example, K^+ in the presence of valinomycin or a lipophilic cation). Alternatively, the H^+ could be accompanied by an anion (for example, a lipophilic anion). The results of Wikström and Saari (*7*) are consistent with the presence of a significant metabolically induced membrane potential if the assumptions of the study are correct (for example, the assumption that the Nernst equation can be used to calculate a membrane potential at all K^+ concentrations). However, these results do not correspond to a definite proof. The interpretation of Wikström and Saari (*7*), that the spectral shifts in ferric cytochromes aa_3 are due to proton binding to this complex may well be in harmony with a model involving proton shifts alone without involving a membrane potential. Similarly, we have no quarrel with the concept that a diffusion potential induces spectral and fluorescence changes in aurovertin and ferric cytochromes aa_3, respectively, or a conformational change in F_1 (adenosine triphosphatase). We have considerable corroborative evidence for an absence of a significant metabolically induced membrane potential. Using four electrofluorimetric dyes we have obtained evidence that in rat liver mitochondria, the magnitude of the metabolically induced membrane potential change is within the range of 0 to −60 mv (*4*).

We have also conducted a variety of experiments on the decay of the K^+ diffusion potential using the same electrofluorimetric dyes (*4*). The potential, as detected by the fluorescence, decays with time but only in the presence of certain cations (for example, Mg^{2+} or, alternatively, a high concentration of Na^+ or choline). In the absence of these ions the decay is very slow, as it is in other systems such as liposomes and red blood cells (*8*) or Ehrlich ascites cells (*9*). Burckhardt (*9*), for example, selects

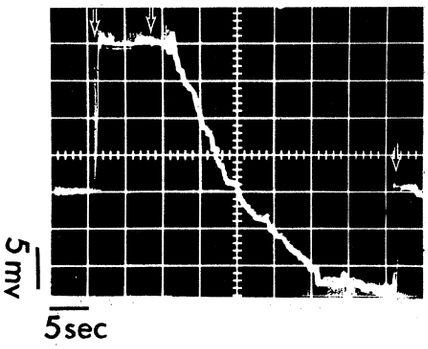

Fig. 1. Except for the use of the electrodes filled with NaCl, this experiment is approximately equivalent to that previously reported (*2*). The arrows indicate in chronological sequence, impalement, addition of valinomycin, and removal of the electrode. Valinomycin was added to the agar overlay about 0.1 mm in thickness to a final concentration of $10^{-7}M$. The medium was maintained at approximately 18° to 25°C. The medium contained 0.30 osmolal sucrose, 1 m*M* tris(hydroxymethyl)-aminomethane, 1 m*M* KCl at *p*H 7.4.

steady state fluorescence as a reflection of membrane potential after 30 minutes.

The stability of the K^+ diffusion potential in the presence of valinomycin results from the fact that the K^+ must leave with an accompanying anion (or in exchange for another cation). A rapid decay from mitochondria would be expected only from considerations based on the chemi-osmotic hypothesis. We regard the lack of a rapid decay as further evidence that the high internal K^+ concentration of mitochondria is not the result of a metabolically induced membrane potential postulated by others.

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highlights of the meeting program for persons with visual impairments; emergency repair service for wheelchairs; round-the-clock telephone service responding to emergency needs; and tour and sightseeing information for handicapped persons. Individuals needing modified hotel accommodations (for example, bathroom door which can accommodate wheelchair, room near elevator, and so forth), interpreting services, or other assistance, are strongly urged to so inform the AAAS Meetings Office (telephone 202/467-4487; TTY users may call 202/467-4497).

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