Table 1. Numbers of times each directional perch was used by a caged white-crowned sparrow (*Zonotrichia leucophrys gambelii*) by day and by night during the period of development of vernal migratory restlessness. The sparrow was a male (28 gm) captured 5 Feb. 1959 near Gilroy, about 30 miles southeast of San Jose State College. Prenuptial molt was completed about 27 April. Critical weights were as follows: 12 April, 31 gm; 18 April, 32 gm; 25 April, 38.5 gm; and 3 May, 38.5 gm. All nights were partly cloudy except that of 25-26 April, which was cloudy and yielded 0.7 in. of rain.

Date (1959)	Orientation of daylight activity								Orientation of nighttime activity							
	E	SE	S	SW	W	NW	N	NE	E	SE	S	SW	W	NW	N	NE
23 Apr.	37	45	54	76	61	49	56	51	1	0	5	3	5	2	1	0
24 Apr.	73	109	154	155	148	131	113	109	0	0	0	5	0	0	0	0
25 Apr.	238	183	241	210	203	234	211	167	0	0	2	1	0	4	0	0
26 Apr.	130	385	314	481	390	295	437	220	31	7	13	31	26	28	66	82
27 Apr.	32	68	53	68	53	55	76	41	17	1	6	19	23	199	477	499
28 Apr.	46	55	121	141	41	78	138	55	4	5	7	12	9	58	1620	186
29 Apr.	24	31	105	373	67	52	242	35	10	12	98	57	15	80	1005	73
30 Apr.	16	39	41	88	55	51	66	30	22	6	5	26	11	18	945	135
1 May	25	64	39	84	48	50	59	49	8	2	2	6	6	22	1185	78
Total	621	979	1122	1676	1066	995	1398	757	93	33	138	160	95	411	5299	1053
Percentage	7	11	13	20	12	12	16	9	1	0	2	2	1	6	73	15

orientation has materialized or (ii) may be necessary to permit the development of orientation. The fall-off in the intensity of daytime activity when nocturnal activity becomes strong also appears to be characteristic of individual birds which exhibit the clearest patterns of orientation.

In August, September, and October (1958), birds which had completed their postnuptial molt and which had gained sufficient weight exhibited significant night restlessness. They showed a significant tendency to move toward the south, southeast, or southwest during the hours of darkness when the sky was clear. Daytime activity tended to be random or somewhat northerly in orientation. It should be noted that these birds were already within a few miles (10 to 50) of their natural winter home, and that this would tend to lead to a more diffuse pattern of activity. The greater strength of the northerly orientation in the vernal period than of the southerly orientation in the estival period was to have been expected, for the birds were many hundreds of miles south of their breeding range.

The presence or the absence of the masonite screen seemed to have little effect on the orientation of day or night activity. Rotation of the screened cage in which a bird showed strong orientation of activity revealed some influence of points of reference in the cage. After a 90° rotation, nearly complete correction was accomplished the first night if the night was clear. If, however, the sky was overcast or partly cloudy, correction was not accomplished as readily.

These results and additional preliminary findings from manipulation of daily photoperiods suggest that the activity-orientation cage provides a useful tool for the study of the physiology of the orientation of migration with birds of the genus *Zonotrichia*.

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State of Dynamic Equilibrium in Protein of Mammalian Cells

Abstract. Labeled strain L cells in suspension tissue culture showed no degradation of protein when maintained in logarithmic growth. Although the protein of these cells was not in dynamic equilibrium, the conclusions cannot be transferred to the intact mammalian organism.

The concept of "dynamic equilibrium" of cellular proteins has been accepted since the investigations of Schoenheimer and his colleagues (*1*). Recently this concept has been challenged by workers studying adaptive enzyme formation in bacteria (*2*). They found that preformed cellular protein did not contribute to a newly induced adaptive enzyme. Furthermore, once an adaptive enzyme was formed and the inducer was removed, that particular enzyme did not incorporate labeled amino acid into its structure. Similar results on the lack of protein turnover were obtained with yeast cells maintained in logarithmic growth (*3*). However, using mammalian tissue culture cells, one group found a turnover of 0.85 to 1.0 percent per hour (18.5 to 21.3 percent per day) (*4*), and other investigators reported a turnover of 12.9 percent per day (*5*).

It is now possible to grow mammalian cells in a manner very similar to that in which bacteria are grown (*6*). By maintaining L cells in logarithmic growth, it has been shown that both deoxyribonucleic acid and ribonucleic acid undergo no turnover in rapidly growing cultures (*7*). These facts have made it desirable to reinvestigate protein degradation in mammalian cells maintained in strict logarithmic growth.

Strain L cells were grown in 250-ml erlenmeyer flasks placed on a rotary shaker in an incubator maintained at 37.5°C. Each flask contained cells (200,000 per milliliter), Eagle's basal medium (72 ml), horse serum (8 ml), penicillin (100 units/ml), streptomycin (100 μg/ml), and leucine-1-C^{14} (3 to 4 $\times$ 10^6 counts per culture, specific activity 1.4 mc/mm) (*8*).

The culture was allowed to grow for 3 days until a high cell number (1 $\times$ 10^6 cells per milliliter) was reached. All counts were made in duplicate on a standard hemocytometer. At this time, one-third of the cells were removed and washed 3 times by centrifugation in 50 ml of Krebs-Ringer phosphate buffer. After resuspension in fresh unlabeled medium, the cells again grew logarithmically and showed a generation time of 26 hours. During this time the cells eliminated almost all the free labeled amino acid from their free intracellular pool. At the end of 3 days, 20 ml of this culture, containing approximately 1.5 $\times$ 10^6 cells per milliliter, was poured into a new flask containing 60 ml of fresh medium. This fresh medium had previously been warmed to 37.5°C, and the *p*H adjusted to 7.1. It was essential that the transfer be completed quickly, carefully, without change in *p*H or temperature, and without other disturbances which might result in compensatory equilibratory reactions and interfere with the delicate autoregulatory processes associated with logarithmic growth of the cells.

Aliquots (5 ml) were removed daily from those cultures maintaining a generation time of 26 hours. The cells were washed three times in 15 ml of Krebs-Ringer phosphate buffer solution by centrifugation at 3000 rev/min. The protein of the centrifuged cells was precipitated with 5-percent cold trichloroacetic acid. After centrifugation, the trichloroacetic acid in the protein-free supernatant was extracted three times with ether, and the remainder, containing the free amino acid pool, was plated on planchets. The trichloroacetic acid precipitate, containing the cellular protein, was extracted twice with ethanol: ether (1:1) at 48°C for 45 minutes and once with hot trichloroacetic acid at 90°C for 1 hour; it was washed with ether and plated on previously weighed planchets. All planchets were counted in duplicate in a windowless gas-flow counter and corrected for self-absorption when necessary.

The results (Table 1) show that the counts per minute in the protein obtained from 1 ml of culture fluid remained constant during the period of the experiment, regardless of the large increase in cell number and in cell weight, and indicate that turnover of radioactive leucine during this period was negligible. It should be emphasized that during this period the cells were suspended in medium containing no labeled leucine and were in logarithmic growth. This is most consistent with the hypothesis that there is no degradation of cellular protein during periods of active growth. Other evidence concerning the lack of protein breakdown was found in the fact that labeled leucine was not found either in the extracellular or in the intracellular free amino acid pool except in negligible quantities. There was a possibility that cellular protein could be degraded and resynthesized without passing through the free pool, but it seemed unlikely that this could have occurred without any appreciable loss of labeled leucine from the cellular protein to the large volume of intra- and extracellular fluid. The relatively enormous quantity of unlabeled leucine present in the intracellular and extracellular fluid would be expected to act as a "trap" for any free labeled leucine degraded and separated from the protein. Oxidative and other degradative reactions did not occur to any appreciable extent. The experiments were not started until after 3 days of preliminary incubation in unlabeled medium, which insured that the amount of label in the free amino acid pool was negligible. It is possible that some proteins which had a very rapid turnover lost their label during this period, and that the constant daily counts represented only the stable residual proteins.

Table 1. Degradation of protein in strain L cells in rapid growth.

Experiment No.	Day No.	No. of cells per ml $\times 10^3$	Cell protein (μg/ml)	Cell protein (count/min ml*)	Cellular free pool (count/min ml†)	Extracellular medium (count/min ml‡)
1	1	250	75	1633	4	20
1	2	640	210	1630	5	31
1	3	1300	450	1600	7	29
1	4	2000	650	1623	6	28
2	1	500	165	1781	9	15
2	2	900	300	1772	5	15
2	3	1700	540	1714	5	20
3	1	200	60	884	2	25
3	2	380	130	884	0	23
3	3	800	280	847	0	29
3	4	1700	560	880	0	24
4	1	420	138	860	3	31
4	2	730	248	850	2	22
4	3	1600	527	840	4	26
5	1	350	120	841	4	30
5	2	620	200	864	0	37
5	3	1200	396	821	5	33

* Count/min in trichloroacetic acid precipitate of washed cells from 1 ml of culture fluid. † Count/min in trichloroacetic acid supernatant of washed cells from 1 ml of culture fluid. ‡ Count/min in 1 ml of culture fluid after removal of cells. These counts represent contaminating extracellular label carried over in the transfer of medium and cells, as previously described, into the final experimental subculture.

These experiments, however, provide evidence that mammalian tissue culture cells maintained in logarithmic growth behave in a manner similar to the behavior of bacteria and yeasts and exhibit no "dynamic equilibrium." It should be strongly emphasized that these cultures are maintained under strictly regulated controlled conditions, and that cultures grown under conditions that allow even minor alterations in the environment for very short periods of time show large fluctuating compensatory changes in the amino acid pattern. The wide range of protein turnover values already reported (12.9 to 21.3 percent per day) (*4*, *5*) may well indicate the variation to be expected when extreme precautions are not taken to avoid equilibratory reactions.

The relevance of these and of previous experiments (*2*, *3*) to the conditions existing in the intact mammalian organism is uncertain. The cells of most organs, except perhaps skin, hematopoietic tissue, and intestinal and urinary tract mucosa, are not in constant division and thus might not be expected to show results similar to cells maintained in logarithmic growth in culture. An active "dynamic equilibrium" has been found in the proteins of "resting" cultures of yeasts, bacteria, and mammalian cells. However, we feel that the term *resting culture* is a euphemism for "dying culture." These cells undergo vast visible and chemical changes in a matter of hours, or at most of days, and are in no way comparable to cells of the liver, heart, kidney, and brain. These latter cells, with negligible mitotic indices, exist for weeks, months, or years without apparent change.

These experiments support the hypothesis that preformed protein does not incorporate new amino acids into its internal structure. We would speculate that in cells whose energies are wholly directed toward self-replication, there is no dynamic equilibrium, while in cells with other, various obligations, and with shifting substrates and changing environments, there is constant degradation and resynthesis of protein to meet these varying demands. In this sense there probably exists a very real dynamic equilibrium (*9*).

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