Biochemical Changes with Age in the Lenses of White Mice

CHANGES with age in the relative amounts of water soluble and insoluble proteins have been studied in cattle, humans and laboratory rats. The changes apparently result from oxidation of cysteine in the soluble portion and its incorporation as cystine in the insoluble protein[1].

Dische *et al.*[1] found that the soluble fraction of rat lenses increased until shortly after the first year and then decreased to the end of life. The insoluble protein increased during the first 900 days of life, then apparently levelled off. Discrete age classes were not used, and no statistical analysis was given. In an attempt to develop a more accurate technique for estimating ages of wild mice than was available, we examined comparable changes in white mice, using a colorimetric procedure. We report here findings for the first 9–10 months of life.

Eighty-eight white mice (*Mus musculus*) of known age (0·5–10 months) were obtained commercially. Every lens used was freed of any adhering vitreous material, ground up in a glass homogenizer, and suspended in 2 ml. of demineralized, distilled water. Soluble and insoluble fractions were separated by cold centrifugation at 12,800*g* for 30 min. The supernatant, soluble fraction was treated with 4 ml. of 10 per cent trichloroacetic acid. The resulting precipitate was recovered by centrifugation as already described. Sediments of soluble and insoluble fractions were dissolved in 1 ml. of 1 N NaOH and analysed for tyrosine, using the method of Lowry[2] as described by Litwak[3]. All daily measurements were calibrated to fresh standard solutions of tyrosine.

The right and left lens from each mouse was processed and analysed separately; subsequently, the mean values of soluble and insoluble fractions per mouse were used for statistical analysis with Student's *t* test. Within each age group, data from the two sexes were combined because there were no statistically significant difference between them.

The soluble fraction increased until the seventh month, with statistically significant differences between successive age groups. After 7 months, there was a significant decrease in this fraction ($P = 0{\cdot}05$). Appropriate statistical data are given in Table 1 for possible future comparison with other species or populations.

Table 1. CHANGES IN THE SOLUBLE FRACTION WITH AGE

	Age in months				
	1	3	5	7	9
Mean	227	354	422	487	450
95% limits	213–241	325–383	390–454	460–514	426–474
Range	152–317	296–425	331–497	427–563	360–528
Standard deviation	40	38	48	45	51
No.	36	9	11	13	19

Changes are expressed as μg of tyrosine.

Table 2 shows comparable figures for the insoluble fraction. This component increased throughout the first 9 months. There was no significant change between the seventh and ninth months, but all other successive age groups showed significant increases ($P = 0{\cdot}05$). The regression of a logarithm of insoluble tyrosine on the logarithm of age is linear. In logarithmic units, the Y intercept of this line is 1·673 and the slope is 0·696.

The ratio of soluble to insoluble fractions decreased throughout the age span studied (Table 3). Differences between successive age groups were statistically significant ($P = 0{\cdot}05$). The rate of change of this index is linear on logarithmic scales. The slope is negative and equals 0·4236, and the Y intercept is 0·6651.

Table 2. CHANGES IN THE INSOLUBLE FRACTION WITH AGE

	Age in months				
	1	3	5	7	9
Mean	51	110	176	247	253
95% limits	47–55	102–118	148–204	230–264	242–264
Range	30–85	97–129	106–233	202–293	213–304
Standard deviation	12	10	42	29	24
No.	36	9	11	13	19

Changes are expressed as μg of tyrosine.

Table 3. CHANGES IN THE RATIO OF SOLUBLE TO INSOLUBLE FRACTIONS WITH AGE

	Age in months				
	1	3	5	7	9
Mean	4·7	3·2	2·5	2·0	1·8
95% limits	4·3–5·1	2·9–3·5	2·1–2·9	1·9–2·1	1·7–1·9
Range	2·7–7·6	2·9–4·1	1·7–3·1	1·6–2·2	1·5–2·0
Standard deviation	1·24	0·39	0·56	0·16	0·14
No.	36	9	11	13	19

Results are expressed as μg of tyrosine.

It is apparent that lens changes in *Mus* are similar to those reported for *Rattus norvegicus*[1] at least for the first 9 months. In rats, the soluble fraction increased rapidly for the first 13 months, then decreased. The insoluble portion probably increased throughout the first 9 months, but the rate of increase was slower near the end of this time. The apparently continual decrease in the ratio of the two fractions was rapid at first, but later became much slower. This ratio in 5 and 10 month old rats, measured in mg of protein (not tyrosine), was approximately 2·4 and 1·7, respectively (computed from Table 1 in ref. 1). Thus, despite large differences in lens size, the relative proportions of the two fractions are practically identical for rats and mice of similar ages.

Information on lens changes in older mice is lacking, but one would expect changes comparable with those seen in rats. The technique seems to be promising for determining age in small mammals.

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Obtaining Synchronous Cultures of Algae

A COMMUNICATION from Lafeber and Steenbergen[1] described a "simple device for obtaining synchronous cultures of algae". They stated that *Scenedesmus obliquus*, *Ankistrodesmus falcatus*, and *Chlorella vulgaris* all underwent synchronous cell division induced by the photoperiod used.

We wish to point out that the three species used are atypical of algae that might be used in synchronous culture experiments, in that they produce autospores rather than undergoing simple cell division. For example, *Scenedesmus* has a coenobial habit, each cell being capable of forming daughter coenobia by longitudinal and transverse divisions of the protoplast to form two, four, eight, sixteen or thirty-two autospores. *Ankistrodesmus* reproduces by forming two, four or eight autospores, and *Chlorella* reproduces by forming two, four, eight or sixteen autospores[2]. This is in contrast to algae which always form two new cells by cell division such as diatoms and dinoflagellates.

We believe that the practice of diluting cultures at the end of each dark period with fresh culture media is questionable. This procedure could cause the cells to enter a new lag phase. Thus, when internal changes in