CHANGES IN HEAD PROTEINS OF *SARCOPHAGA PEREGRINA* WITH AGE

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1. Introduction

One of the characteristic changes associated with aging in cells is an increase in altered molecules [1]. Such alterations have been demonstrated in certain enzymes in cultured human fibroblasts [2–4]. Such alterations of protein molecules can be partly explained by the error catastrophe theory [5], but the possibility of post-translational modification cannot be excluded [6–8]. Most studies on aging have been on changes in certain characteristics of enzymes, such as heat-sensitivity [9,10], but clear evidence of qualitative and quantitative differences between the proteins in young and aged cells has not yet been demonstrated. There seem to be two main reasons for this:

(i) Suitable experimental materials are difficult to obtain, since it is desirable to use post-mitotic cells for such studies;

(ii) Appropriate analytical methods were not available.

However, the method of two-dimensional gel electrophoresis developed in [11], by which it is possible to detect spots containing $\geqslant 0.03$ μg protein, can be used now for this purpose.

This paper describes analyses of changes in head proteins of the flesh-fly, *Sarcophaga peregrina*, with age by two-dimensional gel electrophoresis. The life-span of this fly is ~35 days at 27°C, which is convenient for studies on changes of protein with age. Moreover, the head of flesh-fly is mainly composed of post-mitotic cells. Results showed that certain head proteins present in newly emerged flies disappear rapidly with age, while a few specific proteins tend to accumulate with age, although the total amount of head protein gradually decreased.

2. Materials and methods

2.1. *Animals*

About 200 *Sarcophaga peregrina* adults were kept in a stainless steel cage (30 × 30 × 30 cm) in a light room at 27°C and given dry milk, sugar cubes and fresh water. Under these conditions the flies showed 90% motility in 35 days.

2.2. *Extraction of proteins*

Three flies were decapitated and their heads were ground in a small glass homogenizer with a tightly fitting glass pestle. Then 60 μl solution of 9.25 M urea, 1.5% (w/v) Nonidet P-40, 5% (v/v) β-mercaptoethanol and 2% (w/v) Ampholine (LKB, pH 3.5–10) was added and the mixture was homogenized thoroughly at room temperature (20°C). The homogenate was centrifuged for 10 min at 10 000 × *g* and the resulting clear supernatant was subjected to electrophoresis and protein determination by the Lowry method [12].

2.3. *Polyacrylamide gel electrophoresis*

Two-dimensional gel electrophoresis was carried out as in [11]. Ampholine (pH 3.5–10) was used to make a gradient of pH 4.0–8.0 for electrophoresis in the first dimension in a cylindrical gel (0.2 × 13 cm) containing 5% (w/v) acrylamide. The second dimension slab gel (1 mm thick) contained 10% (w/v) acrylamide and 0.1% (w/v) SDS. Samples of 20 μl, containing 150 μg protein, were applied to each gel. After electrophoresis, the gels were stained with Coomassie brilliant blue R250 by the method in [13].

3. Results

First the amount of head proteins of male flies of various ages solubilized with Nonidet P-40 and urea was measured. Since 80–90% of the proteins are solubilized by this treatment [14], it is possible to calculate the total amount of soluble protein per head. As shown in fig.1, there seem to be two phases of decrease in the amount of head protein with age: rapid decrease for the first 6 days and then slow steady decrease which continues even through the senescent stage, attained by keeping the flies for >30 days. The amount of head proteins in 32-day-old flies was calculated to be 60% of that in newly emerged flies. This decrease was not the result of change in solubility of the proteins with age, because the efficiency of solubilization of head proteins did not change with age. The ratio of the amount of solubilized protein to the weight of heads was always constant irrespective of the age.

Next the proteins solubilized from the heads were analyzed by two-dimensional polyacrylamide gel electrophoresis. More than 400 spots were detected on the gel on applying 150 μg protein, the maximum amount with which good resolution could be obtained under these experimental conditions. Typical electrophoretic patterns obtained with proteins from flies of various ages are shown in fig.2. Comparison of (a) and (d), shows several clear differences between the proteins of newly emerged flies and those of 15-day-old flies. The proteins of newly emerged flies include 3 distinct proteins that are missing from the proteins of older flies. These spots are numbered 1–3. Spots 1 and 2 are large, indicating that they are spots of major component of newborn flies. Spot 1 disappears on day 3, and spots 2 and 3 disappear on day 6, as shown in (b) and (c). Spots of other proteins also decrease with time. For instance, spot 4 in (a) seems to be a huge single spot. This spot actually contains 3 different proteins that can be separated from each other as shown in (d), but since the amounts of these proteins are relatively more in young flies than in older ones these proteins of young flies migrate together as a single dense spot. On day 32 these 3 spots are much fainter than on day 15, as shown in (f). The amounts of protein in spots 5–7 also clearly decrease in older flies.

On the contrary, several proteins seem to accumulate with age. For instance, the apparent intensities of spots 8 and 9 are not significantly different in newly emerged flies, but the intensity of spot 8 is clearly more than that of spot 9 in the proteins isolated on day 15. Since the intensity of spot 9 does not change during aging, this change indicates that the protein in spot 8 accumulates with age: although this protein is present in newly emerged flies it clearly accumulates after day 6. Spots 10 and 11 are also those of proteins that accumulate during aging. These results were obtained with male flies; exactly the same results were obtained with female flies, showing that the decrease in head proteins with age is not sex-dependent. From these results it is evident that head proteins solubilized from adult flies of *Sarcophaga peregrina* with Nonidet P-40 and urea change both in quantity and quality with age. The biological significance of these changes is unknown.

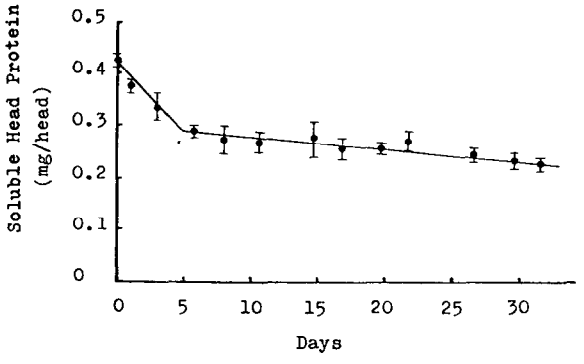

Fig.1. Decrease in head proteins with age. At the times indicated 3 male flies were decapitated and the amount of soluble head proteins was measured. Each point is the average of duplicate measurements.

4. Discussion

It is clear from these results that 3 distinct head proteins disappear at an early stage of adult life and that several proteins decrease in quantity with age. The decrease in quantity of these specific proteins may explain the rapid decrease in total head proteins in the first 6 days after emerging. The later slow steady decrease in the amount of head proteins with age may be due to gradual decrease in the total cell number, since the head of adult flies consists mainly of post-mitotic cells.

With regard to the qualitative changes in the electrophoretic patterns of head proteins, it is possible

that some proteins become insoluble or more soluble during aging, although the efficiency of solubilization of total proteins does not change significantly. It is also possible that certain proteins are selectively degraded and others are selectively accumulated in the cells with age, although the precise molecular mechanism for this is also unknown. Thus, the observed changes may result from a combination of two phenomena: change in cell number and change at a subcellular level. These clear changes with age should also be detectable in other tissues mainly consisting of post-mitotic cells.

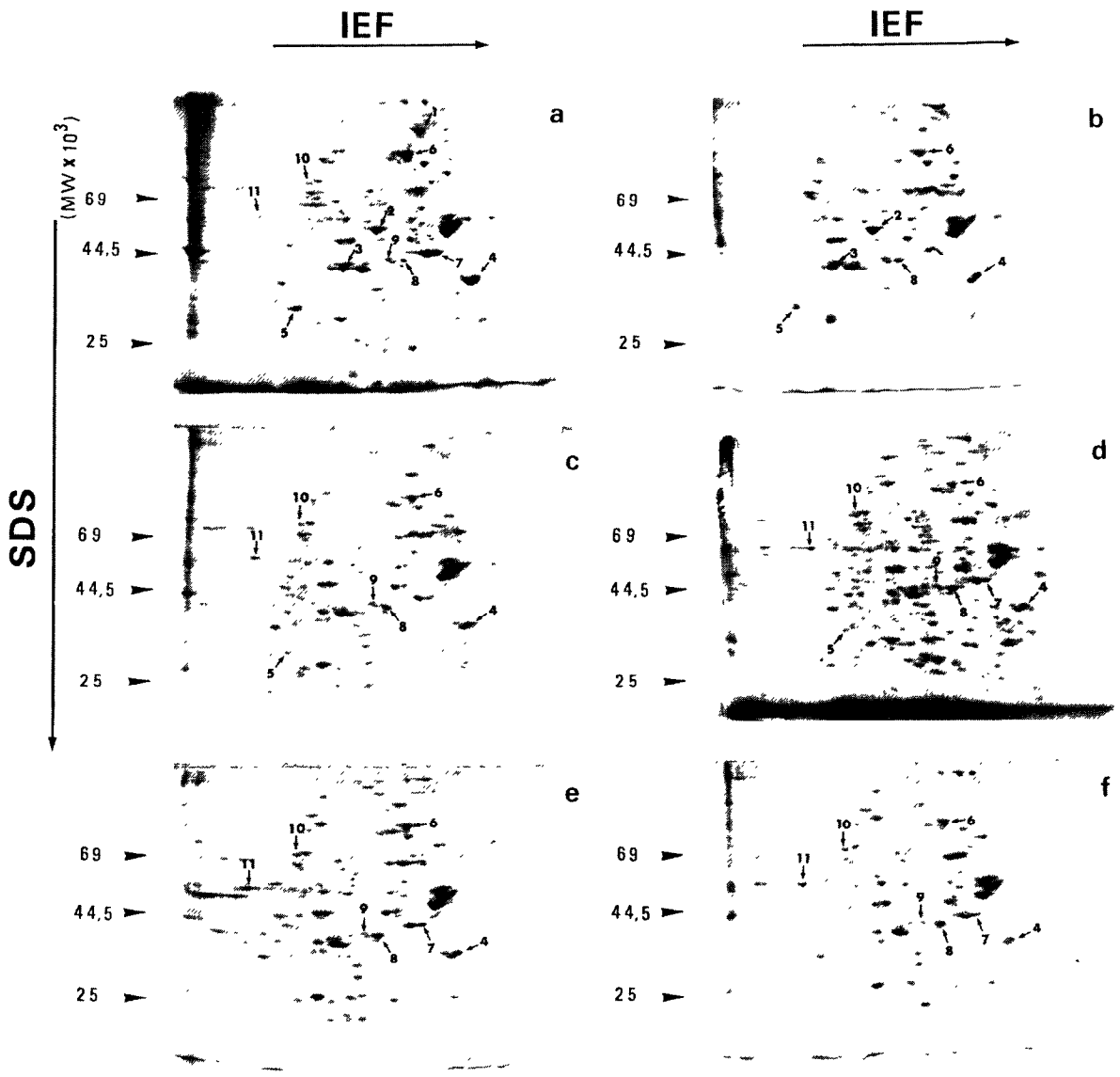

Fig.2. Change in the electrophoretic profile of heat proteins with age. Samples of 20 μl, containing 150 μg protein, were applied to each gel. The gels were calibrated with (mol. wt): bovine serum albumin (69 000); ovalbumin (44 600); α-chymotrypsinogen (25 000). Age of flies: (a) newly emerged; (b) 3-day-old, (c) 6-day-old; (d) 15-day-old; (e) 20-day-old; (f) 32-day-old. For explanation of arrows, see text.

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