fading, the image would reappear after some indefinite period of time. The nature of its reappearance was often total, that is, the image would regenerate to its normal state as a complete pattern. All subjects also reported that partial regenerations of the image might occur, and that these might feature the reappearance of single or parallel straight lines as units. (*e*) The segment of the target least likely to disappear was in the neighbourhood of the fixation point; the more peripheral the part of the pattern, the more likely it was to disappear. (*f*) After about 3 min, subjects reported a tendency for filling in of the whole of the enclosed part of the target. In due course detail was found to reappear in full. Similar results have been obtained with stabilized retinal images[5].

It has been found that, with vivid after-images of geometrical outline shapes, a range of disappearance and reappearance phenomena is experienced comparable with that reported from studies utilizing mechanical stabilizing systems. Of particular interest is the finding that disappearances may be structured, and that a large number of reappearance effects are only partial.

Yarbus[11] and Barlow[7] have suggested that a perfectly stabilized image will suffer total and lasting disappearance and that regeneration of the image must be a consequence of mechanical lens destabilization. While it is not disputed that contact lens slip will induce reappearance of an image, it now seems most likely that the partial and structured reappearances of images reported in this and other[3] studies cannot be attributed solely to such movement. Theories of retinal function that predict permanent disappearance of stabilized images will therefore need reconsideration.

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MISCELLANEOUS

Thin-layer Chromatographic Separation and Quantitative Determination of Several Free Amino-acids of Avian Liver

Thin-layer chromatographic procedures are used widely and accepted as efficient and rapid means of separating numerous organic compounds on a micro scale. The following procedure is presented to illustrate the thin-layer chromatographic separation and rapid quantitative determination of several free amino-acids of avian liver tissue.

Table 1. Recovery of Amino-Acids in a Standard Reference Solution

Amino-acid	Present	Found	Recovered
	μg/3 μl. solution		(%)
Lysine	0·30	0·30	100
Histidine	0·30	0·28	93
Arginine	0·30	0·31	103
Aspartic acid	0·30	0·32	106
Alanine	0·30	0·32	106
Valine	0·30	0·27	90
Leucines	0·30	0·28	93

Table 2. Several Free Amino-Acids of Avian Liver

Chick No.	Plate No.	No. of samples	Lysine	Histidine	Arginine	Aspartic acid	Alanine	Valine	Leucines
			← (μg/3 μl. extract) →						
20	1	6	0·41 ± 0·013*	0·29 ± 0·018	0·33 ± 0·013	0·56 ± 0·056	0·40 ± 0·039	0·28 ± 0·032	0·45 ± 0·031
20	2	6	0·48 ± 0·017	0·31 ± 0·025	0·38 ± 0·014	0·60 ± 0·049	0·41 ± 0·025	0·27 ± 0·028	0·45 ± 0·033
43	1	1	0·51	0·24	0·34	0·50	0·62	0·45	0·67
43	2	1	0·57	0·24	0·38	0·45	0·54	0·45	0·70
52	1	1	0·65	0·30	0·54	1·10	0·77	0·65	1·03
52	2	1	0·62	0·30	0·56	0·75	0·77	0·65	1·00

* Standard error of mean.

Avian liver samples are homogenized in cold distilled water (1 : 4, w/v). Free amino-acids are separated according to Awapara[1] by precipitating the proteins with absolute alcohol and separating the aqueous and organic solvent layers with chloroform.

TLC layers are prepared fresh on glass or plastic[2] plates according to standard procedures, using silica-gel *G* (Brinkmann Instruments, Great Neck, New York). When dry, the plates are placed on a heating device and warmed to 65° C and then spotted with the amino-acid solutions. A total volume of 3 μl. for each spot has been found to be appropriate. A standard reference solution of amino-acids is spotted between each of 2 unknowns. With this procedure a 20 × 20 cm plate is satisfactory for 3 standards and 6 unknowns.

Chromatographic glass tanks, of a type that can be sealed, are kept in a constant temperature atmosphere of 53° C. After the plates are spotted they are placed immediately in tanks containing a mixture of *n*-butanol-acetic acid and water (60 : 20 : 20, v/v/v). Each plate is placed in a separate tank. The solvent is allowed to run 160 mm. The plates are then removed, thoroughly dried, and re-run in the same direction under the same conditions; however, this time a 75 per cent phenol (chromatographic grade) is used as the solvent. The plates are dried again, stained with 0·25 per cent (w/v) ninhydrin (triketohydrindene hydrate) in acetone, and set in the dark for a standardized interval until the colour has developed. The chromatograms are then scanned at 525 mμ in a thin-layer chromatography densitometer (Photovolt Corp., New York).

The recovery of 7 amino-acids in a 3-μl. standard solution is shown in Table 1. Table 2 gives the content of 3-μl. extracts of several free amino-acids of avian livers. Experimental errors related to the analysis of micro quantities of biological materials are well known. Variability can be lessened and reproducibility increased if particular stress is placed on standardizing environmental conditions, preparing uniform plates, obtaining clear-cut separations and sufficient density and constancy of colour development for the particular areas to be scanned.

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