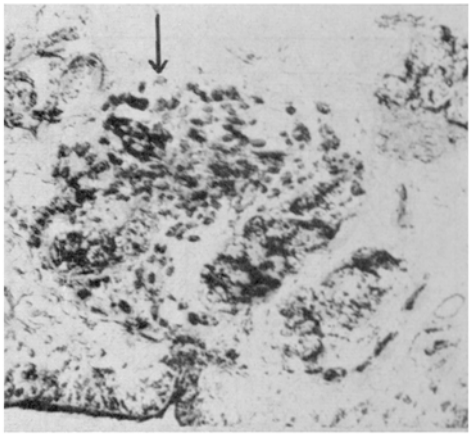

Fig. 2. – Section of *F. hepatica* showing Mehlis' gland (↓) positive to Sudan black B. Fixation in formaldehyde-calcium and postchromation

The shell and its precursors are negative to all the phospholipid tests except aqueous azur I, azur I-Schiff, and Nile blue when material fixed in Bouin's was used. With formol-calcium or Bouin's fixed specimens subsequently deaminised the shell material at all stages remained refractory to the azur and Nile blue tests. It is thus apparent that these stains react with $-NH_2$ groups of shell material since these groups are blocked by formaldehyde[6,7] or removed when deaminised. Nuclei of cells stained by the azur technique may well rely upon phosphoryl radicals as suggested by LOVE and LILES[8] for toluidine blue-molybdate. This would also account for the non-impairment of the reaction of Mehlis' gland cells to azur and Nile blue tests even after deamination.

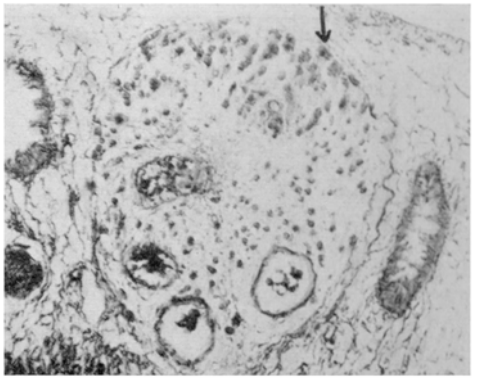

Fig. 3 – Section of *F. hepatica* showing Mehlis' gland (↓) negative to Baker's acid hematein. Fixation in weak Bouin's followed by pyridine extraction

Hence neither the egg-shell at any stage nor its precursors give phospholipid reactions, whilst Mehlis' gland secretion is positive to all the tests. Conversely there is no indication whatsoever that Mehlis' gland secretion is a participant in the tanning system that is so characteristic of egg-shell formation. Thus the secretion would probably be connected with some mechanism effecting the release of egg-shell precursors from the vitelline cells but does not itself form part of the shell. It is tempting to compare this mechanism with the process of hemolysis where lysolecithin derived enzymatically from lecithin serves as a powerful hemolytic agent[9]. It could be suggested here that Mehlis' gland secretion may be closely related to lecithin and is converted by an enzyme system into an agent capable of effecting release of the shell precursors from the vitelline cells, but these possibilities are the subject of further research.

Thanks are expressed to the Government of the United Kingdom for a scholarship under the Colombo Plan. Grateful acknowledgments are due to Prof. E. A. SPALL for facilities, encouragement, and criticism, and to Professor J. D. SMYTH for help and suggestions while I enjoyed the hospitality of his laboratory at Trinity College, Dublin. I am indebted to Dr. T. KERR for much helpful discussion.

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Zusammenfassung

Histochemische Untersuchungen der Mehlischen Drüse von *Fasciola hepatica* erweisen deren Phospholipidabsonderung. Es wird auf die Möglichkeit einer engen Beziehung des Phospholipids zu Lezithin und seine enzymatische Aktivierung zur Herauslösung von Eischalenprokursoren hingewiesen.

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[9] J. A. LOVERN, *Chemistry of Lipids of Biochemical Significance* (Methuen & Co. Ltd., London 1957).

Two New Pteridinic Pigments in *Drosophila melanogaster*

From the compound eye of *Drosophila melanogaster*, LEDERER[1] extracted a red pigment, which was named drosopterin since it was considered to be a pteridin derivative. Further studies showed that the red pigment consists of a number of chemically related pteridines. By talc column chromatography, MAAS[2] observed the resolution of the pigment into several coloured layers; HEYMANN *et al.*[3] were later able to separate some components of the red pigment by chromatography on silica gel column.

HADORN and MITCHELL[4] separated by paper partition chromatography a number of fluorescent substances and eye pigment components. Using this technique they studied many eye colour mutants, affecting the inheritance of the red pigment; it was found that many mutant genes block the synthesis of the red pteridines or decrease the amount of pigment fixed in the ommatidia. VISCONTINI *et al.*[5] isolated from wild type flies three compounds, named drosopterin, idodrosopterin, and neodrosopterin; some details on the chemical structure of these substances

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[2] W. K. MAAS, Genetics *33*, 177 (1948).

[3] H. HEYMANN, F. L. CHAN, and C. W. CLANCY, J. Amer. chem. Soc. *73*, 5148 (1951).

[4] E. HADORN and H. K. MITCHELL, Proc. nat. Acad. Sci. Wash., *37*, 650 (1951).

[5] M. FISCONTI, E. HADORN, and P. KARRER, Helv. chim. Acta *40*, 579 (1957).

Table

Substance found in chromatograms	Reference no. on map	Mean *Rf*			Fluorescence color	Identification
		BAW	FA 5%	KCl 20%		
Neodrosopterin	1	0·04	0·12	0·03	red	5
Drosopterin	2	0·07	0·28	0·13	orange	5
Isodrospterin	3	0·10	0·37	0·22	orange	5
Spot-4	4	0·08	0·44	0·31	orange	—
Spot-5	5	0·10	0·47	0·39	orange	—
Iso-xanthopterin	6	0·22	0·38	0·42	purple	A
Xanthopterin	7	0·24	0·52	0·44	green	A
Sepiapterin I	8	0·33	0·46	0·26	yellow	6
Sepiapterin II	9	0·41	0·39	0·18	yellow	7
2-amino-4-oxy-pteridine	10	0·24	0·83	0·76	blue	A
Biopterin*	11	0·28	0·75	0·64	blue	A
Kynurenine	12	0·41	0·83	0·77	pale blue	B
3-oxy-kynurenine	13	0·36	0·75	0·65	yellow	B
Xanthurenic acid	14	0·55	0·46	0·19	pale blue	B
Unknown	15	0·12	—	0·11	red	—

A: gift of Prof. M. VISCONTINI; B: gift of Prof. L. MUSAJO. * 2-amino-4-oxy-6(1-2-dioxy-propyl)-pteridine.
BAW: butanol-acetic acid-water (4:1:5); FA: formic acid.

were obtained[8]. VISCONTINI was recently able to crystallize drosopterin[9].

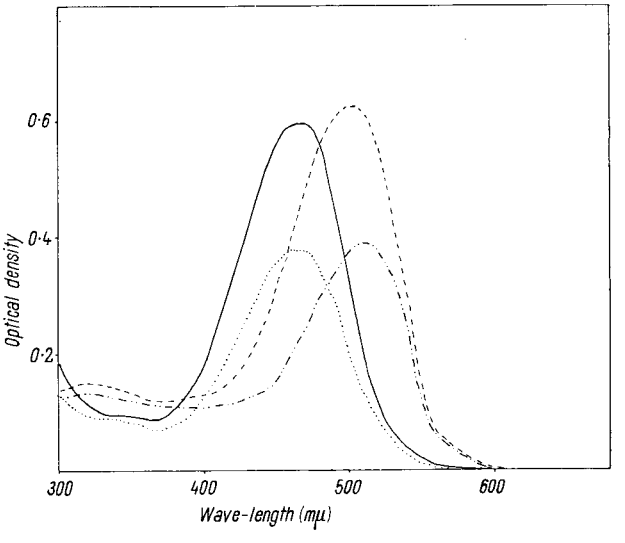
The present paper deals with the components of the red pigment studied by paper partition chromatography; two previously unknown pteridines were found in wild type flies.

Drosophila melanogaster flies were reared on standard corn meal-sugar-agar medium at 25°C, avoiding an overcrowding of the cultures. After emergence the imagoes were collected and aged for ten days before use.

The flies were prepared for chromatography following the technique of HADORN and MITCHELL[4]. Whatman paper No. 1 was used for two-dimensional chromatography; butanolacetic acid-water (4:1:5) or 5% formic acid were used as solvents for the first run, while KCl 20% was found to be the better suited solvent for the second run[10]. Concentrated extracts of the pigments were prepared by homogenizing a large mass of flies (2–3 g) with 10 ml of 80% ethanol containing 1% HCl; after centrifugation, the precipitate was discarded and enough ether (ten times the volume of the supernatant) was added to precipitate the red pigment. Centrifugation allowed the recovery of almost all the coloured material in the precipitate, which was then dried, redissolved in 80% ethanol with 1% HCl and recentrifugated. The clear supernatant was concentrated and analyzed by means of chromatography.

Two-dimensional chromatograms of wild type flies (Oregon R) showed the presence of many weakly fluorescent spots and of five strongly fluorescent and orange-red coloured spots. Most of the substances involved in the fluorescent spots were found to correspond to compounds described previously. The identity of the spots was tested by adding separately to the chromatograms samples of the pure compounds, when available. Three of the orange-red substances were identified by their behaviour (R_f values, fluorescence color) reported in the literature[5], as drosopterin, isodrosopterin, and neodrosopterin (see Table).

Since two of the observed spots did not correspond to the previously described ones, an attempt was made to obtain some information on the substances involved. By chromatography of flies extracts, large quantities of these compounds were separated; the spots were cut from the paper, eluted by flowing 0·1 *N* HCl or 0·1 *N* NaOH and measurements of the absorption spectra were made in a Beckman DU spectrophotometer. In the Figure the absorption curves between 300 and 600 mμ are represented of spot 4-substance and of drosopterin, obtained in the same manner.



Absorption curves of drosopterin (in 0·1 *N* HCl ——, in 0·1 *N* NaOH - - - -) and of the spot 4-substance (in 0·1 *N* HCl ....., in 0·1 *N* NaOH ·—·—·—·)

It seems likely that spots 4 and 5 are made of two pteridinic compounds, structurally related to drosopterin and isodrosopterin. While the absorption spectra of spots 4 and 5 are practically indistinguishable from those of the compounds named, the behaviour (as determined on the basis of R_f values in many solvents) of spot 4-substance

[6] H. S. FORREST and H. K. MITCHELL, J. Amer. chem. Soc. *77*, 4865 (1955).
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[8] M. VISCONTINI, Helv. chim. Acta *41*, 1299 (1958).
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[10] C. E. DALGLIESH, Biochem. J. *64*, 481 (1956).

is very similar to that of drosopterin and that of spot 5-substance to isodrosopterin.

In order to determine the time of pigment formation during metamorphosis, a great number of larvae were raised on standard medium. At the time of metamorphosis the vials were inspected every hour. Only pupae with white puparium were collected and transferred on filter paper moistened with Ringer's solution and kept inside at 25°C. From approximately the 85th h through eclosion time (120 h from pupation) drosopterin, isodrosopterin, and neodrosopterin are laid down in the eyes, while no trace of spots 4- and 5-substances is to be found.

Immediately after emergence of the imagoes, synthesis of spot 4- and spot 5-substances begins. Flies were analyzed by paper chromatography within the hour after emergence; at this time spots 4 and 5 were present. The amount of these compounds then increases considerably during the first hours of imaginal life. The reaction leading to the formation of these two pigments therefore occurs only in imaginal life. Environmental conditions, like temperature, light or darkness, do not seem to affect this reaction to an appreciable extent. No difference in the relative amounts of eye pigments could be observed in flies raised in light or darkness.

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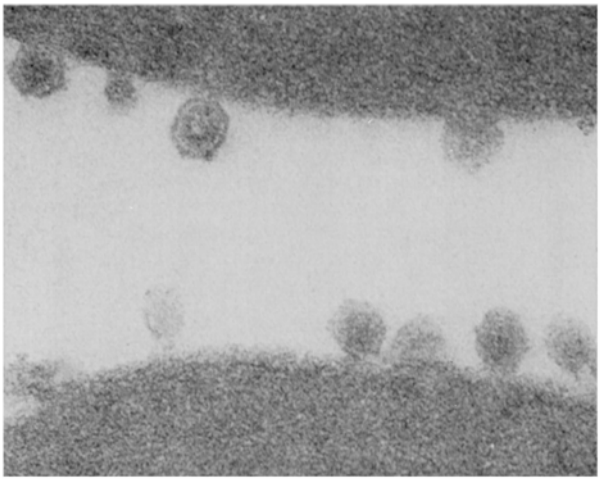
Zusammenfassung

Der extrahierte rote Augenfarbstoff von *Drosophila melanogaster* zeigt papierchromatographisch ein Gemisch von 5 rot-orange fluoreszierenden Pterinen. Zwei von diesen bisher nicht beobachtete Stoffe treten im Puppenstadium noch nicht auf, sondern erst nach dem Ausschlüpfen der Fliegen.

Zur Gestalt des Influenza-Virus

Elektronenoptische Untersuchungen von MORGAN *et al*[1, 2] über die Struktur des Influenza-Virus (Stamm PR 8, Lee und A/Persian Gulf) an der Oberfläche infizierter Zellen zeigten im Ultradünnschnitt sphärische Partikel mit einem Durchmesser von etwa 70 mμ. Das Material war mit 1%iger gepufferter OSO_4-Lösung fixiert und in Methacrylat eingebettet worden. Ebenso beschrieben BIRCH-ANDERSEN und PAUCKER[3] sphärische Partikel von 70 mμ Durchmesser an Suspensionen des Influenza-Virus (Stamm PR 8 des A-Virus). Auch hierbei wurde für die Schnittuntersuchungen mit OsO_4 fixiert und in Methacrylat eingebettet.

Eigene elektronenoptische Untersuchungen am Influenza-Virus (A/Singapur I/57) im Stadium der Hämagglutination erbrachten nach der Fixierung mit $KMnO_4$ eine andere Form. Zur Verhinderung der Abkopplung des Virus von der Erythrozytenoberfläche wurde in Abänderung der von JENSEN und FRANCIS[4] für serologische Belange entwickelten Technik folgende Vorbehandlung angewandt: Ein Volumenteil der Virussuspension (Allantoisflüssigkeit) wird mit 0,5 Volumenteilen einer 0,04 *M* wässerigen KJO_3-Lösung versetzt, nach einstündiger Aufbewahrung bei + 4°C der Flüssigkeit das 1,5fache (des Gesamtvolumens) einer 10%igen Glukoselösung hinzugefügt und bei + 4°C 1/4 h belassen. Die Erythrozyten wurden mit 3%iger Formollösung 48 h bei + 4°C behandelt, danach 6mal innerhalb von 12 h in 0,9%iger NaCl-Lösung gewaschen. Maximal 0,1 cm³ Erythrozytensuspension wurden in 50 cm³ der vorbehandelten Virussuspension aufgeschlemmt. Nach einer 12stündigen Virus-Hämadsorption bei + 4°C erfolgte die $KMnO_4$-Fixierung und Einbettung in «Plexigum»[5]. Eine Kontrastierung der Schnitte kann mit Uranylazetat zusätzlich erfolgen. Als Ergebnis zeigt sich bei etwa der Hälfte aller adsorbierten Elementarkörper im Ultradünnschnitt eine sechseckige Form mit einem Durchmesser von 70 bis 80 mμ (Abb.).



Influenza-Virus (A Singapur I/57) adsorbiert an der Oberfläche formolbehandelter Erythozyten. $KMnO_4$-Fixierung. Vergrösserung 130000

In Analogie hierzu erbrachten Untersuchungen von ANDRES und NIELSEN[6] am bis dahin im Schnitt als rund bekannten Adenovirus nach der $KMnO_4$-Fixierung eine sechseckige Gestalt des Elementarkörpers.

In welchem Masse sich durch die Fixierung mit Kaliumpermanganat andere Strukturen darstellen als nach der OsO_4Fixierung - wie etwa eine verschiedenartige Kontrastierung des Innenkörpers - kann bislang ebensowenig entschieden werden wie die Möglichkeit einer Beeinflussung bzw. Änderung der Gestalt des Influenza-Virus durch die Vorbehandlung der Virussuspension.

Ich danke Herrn Dr. D. PETERS für das fördernde Interesse an der Arbeit, Herrn Dr. E. MANNWEILER für die Virussuspensionen und der Deutschen Forschungsgemeinschaft für finanzielle Unterstützung.

M. E. BAYER

Abteilung für Virusforschung, Tropeninstitut, Hamburg, 16. Juli 1959.

Summary

Influenza-Virus particles are generally considered to be of spherical shape after conventional fixation in OsO_4. However, after fixation with $KMnO_4$, the elementary bodies of the Influenza-Virus (Type A Singapur I/57) - adsorbed on the surface of erythrocytes - exhibit hexagonal shape in ultrathin-sections.

[1] C. MORGAN, H. M. ROSE und D. H. MOORE, J. exp. Med. *104*, 171 (1956).

[2] C. MORGAN und H. M. ROSE, 9. Symposium, Society for General Microbiology, London 1959, p. 256.

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