Host-transplant Interactions in Biosynthesis of

*Drosophila* Pteridines

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**INTRODUCTION**

A series of fluorescent pteridines may be revealed and separated by paper chromatography (Hadorn & Mitchell, 1951) from various organs of *Drosophila* in which they appear in high concentrations. Striking differences in the quantities and in the developmental patterns of these compounds are produced by a variety of gene mutations.

While the chemical analysis of the fluorescent substances is rapidly progressing (cf. Forrest & Mitchell, 1955; Viscontini *et al*., 1955; Viscontini, 1958) the information on the mode and the sites of their biosynthesis is, as yet, extremely scanty. The only well-established reaction involving two of the more abundant pteridines is the enzymatic conversion of 2-amino-4-hydroxy-pteridine (HB\(_1\)) to isoxanthopterin (IX) in the presence of the enzyme xanthine dehydrogenase (Forrest, Glassman, & Mitchell, 1956). The importance of this reaction *in vivo* could be demonstrated by experiments with the mutant rosry (ry) which is devoid of the enzyme (Hadorn & Schwinck, 1956; Hadorn & Graf, 1958; Hadorn, Graf, & Ursprung, 1958). Normalization of IX content in rosry eye-disks or testes transplanted into the abdomen of a wild-type host was found to be correlated with a decrease in the concentration of the precursor substance HB\(_1\). Furthermore, injections of HB\(_1\) into wild-type larvae leads to an increase in the IX content of the developing pupae (Graf, Hadorn, & Ursprung, 1959).

The synthesis of the more complex red eye-pigments (drosopterines = DP; cf. Viscontini, Hadorn, & Karrer, 1957) might be assumed to be closely associated with that of IX, because defects and repairs in both fractions appear to be intimately coupled in the mutants rosry (Hadorn & Schwinck, 1956) and maroon-like (Glassman, Forrest, & Mitchell, 1957). In these genotypes the absence of IX is accompanied by a severe reduction in red pigments, while experimental transfer of eye-tissue to a wild-type host results in concurrent normalization of both deficiencies. It should be noted, however, that other eye-colour mutants like *se* (Hadorn & Ziegler-Günder, 1958) and *Pm* (Ronen, 1957)

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fail to form the drosopterines or contain them in reduced quantities, while IX is present in normal or even increased amounts.

Sepiapteridine (SP) and HB₂ are often increased in genotypes with red-pigment defects (se, ry, Pm) but in other cases (l₂cd; Anders, 1955) they may exhibit a corresponding reduction (Hadorn, 1956). The biosynthetic relationship between these two pteridines and the remaining fluorescent compounds is thus completely obscure.

An experimental attack on the differentiation of gene-conditioned substances is rarely possible where gene and endproduct are confined within the limits of the same cell or tissue. If, on the other hand, biochemical differentiation in an organ is governed extrinsically by means of diffusible products, this distance-steering (or non-autonomy) offers an ideal approach for planned interference. The analysis of pteridine relationships should, therefore, be greatly facilitated if the formation of one or several of these compounds was found to be universally non-autonomous.

Indeed, recent work in this laboratory (Hadorn & Ziegler-Günder, 1958; Hadorn, Graf, & Ursprung, 1958) tends to indicate that the level of isoxanthopterin in transplanted eyes and testes is generally and completely host-dependent. While confirming the non-autonomy of isoxanthopterin formation, the present study exploits this phenomenon in order to establish some developmental relationships between IX and the other pteridines. Changes in supply and demand of IX within the host-transplant system are found to be reflected, as a rule, in the concentrations of other pteridine fractions.

MATERIAL AND METHODS

The stocks of Drosophila melanogaster utilized were sepiaoid (sed; 3–64·5 ±), rosy (ry; 3–51 ±; cf. Hadorn & Schwinck, 1956), white (w; 1–1·5), and the inbred laboratory stock 'Sevelen'. The strain of sed had been backcrossed to Sevelen for nine generations, while ry and w were not coisogenic with the control stock. In view of the higher isoxanthopterin content of male as compared with female eyes (Hadorn & Ziegler-Günder, 1958) the experiments were restricted to males.

Experimental and control animals of all stocks employed were reared on standard sugar-cornmeal-agar-yeast medium at 25 ± 1°C. The transplantations by means of the method developed by Ephrussi & Beadle (1936) were performed on host and donor larvae during the second half of the third instar. Unless otherwise stated, two eye-disks were transplanted into each host, and the dissections for chromatography were carried out on the 6th day of adult life.

The method employed in one-dimensional chromatography has been described in detail by Ziegler-Günder & Hadorn (1958) and by Hadorn & Graf (1958). Optimal separation by ascending chromatography is achieved with a run of 25 to 27 cm. in propanol-5 per cent. ammonia solution (2:1) if the paper is cut into conical strips below each starting-point and suspended for one hour in the
saturated atmosphere of the chromatographic tank before dipping into the solvent.

Hadorn & Graf (1958) summarize the existing information on the composition of the four fractions separated by this procedure. It will therefore suffice to list them briefly, in ascending order of their Rf values:

- **Group I** DP (drosopterin, neodrosopterin, isodrosopterin; Viscontini *et al.*, 1957).
- **Group II** XP (isoxanthopterin, xanthopterin, and a substance related to xanthopterin).
- **Group III** SP (sepiapteridine), for which Forrest & Mitchell (1954) tentatively proposed a structure such as 8-lactyl-7,8 dihydro-2 amino-4-hydroxypteridine-6 carbonic acid, and in chromatograms of some *Drosophila* organs, riboflavine (Viscontini *et al.*, 1955).
- **Group IV** HB (HB\(_1\) = 2-amino-4-hydroxypteridine and HB\(_2\), most probably identical with biopterin).

In group II the fluorometric measurements with the filter systems employed are largely due to xanthopterin. In order to measure isoxanthopterin separately, the chromatograms must be developed in propanol-ammonia 1 per cent. This solvent allows a fair separation of isoxanthopterin and xanthopterin, but the Rf values of SP and HB are so similar with this development that they have to be measured in a single spot.

Two-dimensional chromatography was applied in a few cases. The solvents for the two dimensions were propanol-5 per cent. ammonia (2:1) and butanol-water-acetic acid (20:3:7). In this way it is possible to separate the following compounds: drosopterines, isoxanthopterin, the xanthopterins (xanthopterin and the compound related to it), the SP complex, HB\(_1\), and HB\(_2\).

Owing to development and drying in a darkened room, only negligible amounts of the decomposition product pterine-carbonic acid (Forrest & Mitchell, 1955; Ziegler-Günder, 1956) appeared on the chromatograms. The fluorescence of the spots, traced by long wave UV light, was determined in a fluorometer directly from the dry paper (Hadorn & Kühn, 1953). The primary filter employed has a maximum transmission (m.t.) at 366 m\(\mu\), and the secondary filter system consists of two filters with m.t. at 509 and 578 m\(\mu\), respectively. Where isoxanthopterin was measured alone, the m.t. of the secondary filters was at 509 and 625 m\(\mu\).

**RESULTS**

**A. Effects of inhibited isoxanthopterin synthesis (experiments with sepiaoid)**

The mutant *sepiaoid* (*sed*) was chosen for reciprocal eye-transplantations with wild type because of its pronounced deficiency in isoxanthopterin. Table 1 compares the fluorometric values for adult *sed* eyes with those obtained for wild type. In the brown *sed* eyes the drosopterines are much reduced, while the SP and HB fractions are increased about threefold. Two-dimensional chromat-
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graphy indicates that HB$_2$ (bioppterin) is largely responsible for the rise in the level of the HB group. The XP spot measures slightly more in the mutant. However, visual inspection of the chromatograms by UV light shows that this reading is almost entirely due to xanthopterin, a substance with emerald green fluorescence. In chromatograms of wild type, on the other hand, this spot contains in addition to xanthopterin the isoxanthopterin, which is easily distinguished by its deep violet fluorescence. The level of xanthopterin in sed eyes is therefore somewhat higher than indicated in Table 1. Nawa, Sakaguchi, & Taira (1957) were unable to extract xanthine oxidase from sed animals. However, this enzyme cannot be completely lacking, since the testes of sed males contain a small amount of isoxanthopterin.

It is possible to circumvent the difficulties arising from the overlap of isoxanthopterin and xanthopterin and to obtain fluorometric readings for pure isoxanthopterin by selecting 50-hour old pupae for chromatographic analysis.

### Table 1

*Pteridine groups in sepiaoid (sed) eyes compared with wild type (+)*

The fluorometric values for sepiaoid are expressed as fractions of the mean values for wild type. In this and in all following tables mean values are recorded with ± their standard errors.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of meas.</th>
<th>DP</th>
<th>XP</th>
<th>SP</th>
<th>HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>32</td>
<td>1.00±0.01</td>
<td>1.00±0.02</td>
<td>1.00±0.01</td>
<td>1.00±0.01</td>
</tr>
<tr>
<td>sed</td>
<td>22</td>
<td>0.40±0.01</td>
<td>1.18±0.03</td>
<td>3.44±0.13</td>
<td>2.81±0.10</td>
</tr>
</tbody>
</table>

Hadorn & Ziegler-Günder (1958) have shown that eye-disks of this stage contain considerable quantities of isoxanthopterin, while the remaining pteridines have not as yet accumulated. The effects of reciprocal eye-transplantations between sed and wild type were therefore ascertained, in the first instance, at 50 hours after pupation of the hosts. The results are shown in Table 2. In sed hosts neither sed nor wild-type eyes are able to form more than a trace amount of isoxanthopterin, but both build up considerable quantities in a normal host. The difference between + in + and sed in + is statistically not significant and may not be real.

### Table 2

*Isoxanthopterin content of reciprocal eye transplants between sed and wild type*

Measured 50 hours after pupation of host in arbitrary fluorometric units.

<table>
<thead>
<tr>
<th>Host</th>
<th>+ transplants</th>
<th>sed transplants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluorometric value</td>
<td>No. of meas.</td>
</tr>
<tr>
<td>+</td>
<td>27.2±5.5</td>
<td>6</td>
</tr>
<tr>
<td>sed</td>
<td>1.8±0.5</td>
<td>6</td>
</tr>
</tbody>
</table>
The isoxanthopterin defect characterizing the *sed* genotype is established as non-autonomous in the data of Table 2. The reaction of the other pteridine fractions to changes in isoxanthopterin synthesis had to be examined in transplants dissected from adult hosts.

Table 3a, b summarize the data for normal eye-disk which have developed in the abdomens of wild type and *sed* hosts respectively. Beadle & Ephrussi (1936) observed that the *sed* eye-colour behaves as an autonomous character. Our measurements for the drosopterines are in complete agreement with this statement. Nevertheless, the normal eye is much affected by the *sed* host. The virtual block in isoxanthopterin synthesis is correlated with marked changes in three other pteridine fractions. There is a pronounced increase in xanthopterin, a drop in the sepiapteridine group and an enhanced accumulation of HB. Table 3b is based on two-dimensional chromatograms. In spite of the large variance obtained with this method, it gives some additional information: the net increase in xanthopterin in the *sed* host is seen to be about threefold. Thus, while the *sepiaoid* mutant possesses only a little more of this substance than wild type (cf. Table 1) it is produced in very large quantities by the normal eye in the environment of a *sepiaoid* host. Table 3b also indicates that the rise in the HB fraction involves HB\(_1\) (the known precursor of isoxanthopterin) while HB\(_2\) appears to be decreased.

### Table 3a

**The influence of *sepiaoid* hosts on wild-type eye-transplants**

DP: the mean fluorometric reading for + in + is fixed as unity. XP, SP, and HB: for each spot, the readings of these groups were expressed as fractions of DP readings.

<table>
<thead>
<tr>
<th>+ eye-disks in</th>
<th>DP</th>
<th>XP</th>
<th>SP</th>
<th>HB</th>
<th>No. of meas.</th>
<th>No. of transpl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ hosts</td>
<td>1.00±0.03</td>
<td>1.00±0.06</td>
<td>1.00±0.05</td>
<td>1.00±0.06</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td><em>sed</em> hosts</td>
<td>0.98±0.04</td>
<td>1.65±0.10</td>
<td>0.74±0.04</td>
<td>1.31±0.07</td>
<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>

### Table 3b

**The influence of *sepiaoid* hosts on wild-type eye-transplants (from two-dimensional chromatograms)**

All fluorometric values are recorded as percentage of DP readings of corresponding chromatograms.

<table>
<thead>
<tr>
<th>+ eye-disks in</th>
<th>IX</th>
<th>XP</th>
<th>SP</th>
<th>(\text{HB}_1)</th>
<th>(\text{HB}_2)</th>
<th>No. meas.</th>
<th>No. transpl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ hosts</td>
<td>52.5±4.2</td>
<td>25.3±3.9</td>
<td>27.4±7.0</td>
<td>9.6±1.6</td>
<td>23.9±3.0</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td><em>sed</em> hosts</td>
<td>17.1±2.1</td>
<td>74.9±7.8</td>
<td>16.1±0.8</td>
<td>21.7±5.4</td>
<td>17.2±2.4</td>
<td>4</td>
<td>27</td>
</tr>
</tbody>
</table>

* No visible violet fluorescence in this spot.
The impact of a normal environment on the fluorescent substances of a *sepiaoid* eye is shown in Table 4. The negative correlation between isoxanthopterin synthesis and the levels of xanthopterin and HB which was observed in normal eye-disks (Table 3a, b) is again manifest. However, the *sed* eye fails to show any positive correlation between isoxanthopterin and the SP complex.

### Table 4

*The influence of wild-type hosts on *sepiaoid* eye-transplants*

<table>
<thead>
<tr>
<th>sed eye-disks in</th>
<th>DP</th>
<th>XP</th>
<th>SP</th>
<th>HB</th>
<th>No. of meas.</th>
<th>No. of transpl.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>sed</em> hosts</td>
<td>1.00±0.05</td>
<td>1.00±0.03</td>
<td>1.00±0.02</td>
<td>1.00±0.05</td>
<td>23</td>
<td>46</td>
</tr>
<tr>
<td>+ hosts</td>
<td>0.96±0.04</td>
<td>0.96±0.03</td>
<td>0.96±0.02</td>
<td>0.82±0.03</td>
<td>30</td>
<td>60</td>
</tr>
</tbody>
</table>

These experiments demonstrate that the wild-type organism produces an agent which normalizes the isoxanthopterin level of *sepiaoid* implants without affecting their content in red pigments. It is possible that the material passed by the host to the implant might be identical with the *ry* agent postulated by Hadorn & Schwinck (1956), though the latter supplements the defects in both the isoxanthopterin and the drosopterin fractions of *rosy*. Since wild-type fat-bodies were found (Hadorn & Schwinck, 1956) to constitute one of the sources of this agent, their effects on the isoxanthopterin metabolism of *sed* males were tested. Fat-bodies of the stock ‘altered ratio’ (a.r.) were also injected into *sed* hosts. The a.r. stock exhibits an excess of isoxanthopterin in addition to other changes in pteridine ratios as compared with wild type (Goldschmidt, 1958).

The testis of *sed* in which the isoxanthopterin content is reduced to about one-twelfth of wild type served as a test object. The injections produced a small but significant rise in the IX level of the host testes. There was also an increase in the SP fraction of the experimental testes. It is true that in fat-body injections no more than one-eighth to one-sixth of the total fat-body content of the donor can be transferred. But this dosage problem can hardly account for the large scatter observed in the present experiment as well as in that of Hadorn & Schwinck (1956), in which the effect was more drastic.

### B. Experimental separation between the drosopterin deficiency and the isoxanthopterin block of the *rosy* phenotype

While Hadorn & Schwinck (1956) had demonstrated that a normal host supplies the material required by *rosy* eyes for normalization of isoxanthopterin and of the red pigments, Hadorn & Graf (1958) investigated the repercussions of this repair on other pteridine fractions. The abnormally high SP and HB concen-
trations characterizing *rosy* were observed to drop in a wild environment. Since drosopterines and isoxanthopterin showed correlated changes in this experimental situation, it could not be decided whether they were jointly or separately responsible for the other shifts in the pteridine pattern.

**Table 5**

Relative pteridine fractions in *ry* transplants in normal and in *sepiaoid* hosts

For each spot, XP, SP, and HB readings were recorded as fractions of DP readings. The mean ratios determined for *ry* in + were fixed as unity.

<table>
<thead>
<tr>
<th>ry eye-disks in</th>
<th>XP</th>
<th>SP</th>
<th>HB</th>
<th>No. of meas.</th>
<th>No. of transpl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ hosts</td>
<td>1·00±0·04</td>
<td>1·00±0·05</td>
<td>1·00±0·05</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td><em>sed</em> hosts</td>
<td>1·86±0·11</td>
<td>0·71±0·03</td>
<td>2·04±0·12</td>
<td>15</td>
<td>30</td>
</tr>
</tbody>
</table>

*Text-fig. 1.* Pteridine fractions in *ry* eye-transplants in three different hosts. White columns: in *ry* hosts (based on 20 transplants). Cross-hatched columns: in + hosts (based on 34 transplants). Black columns: in *sed* hosts (based on 30 transplants). The heights of the columns correspond to arbitrary fluorometric values for the four pteridine fractions. The bars attached to the columns indicate the extent of the standard errors of the means.

We have shown that drosopterin differentiation of a normal eye rudiment is not impeded in a *sepiaoid* host, while isoxanthopterin metabolism is severely disturbed. Hence this host should provide suitable conditions for the experimental separation of the two pteridine defects in *rosy* metabolism. The results
appear in Table 5 and Text-fig. 1. It is seen that the sed hosts have corrected the drosopterin defect of ry eyes. Rosy transplants in sed hosts had formed even somewhat more red pigment than those developed in wild type. But this difference should be regarded with caution, since it may be due to discrepancies in developmental rates between the two host strains. The XP fraction of ry eyes grown in sed revealed no isoxanthopterin on inspection under UV light. There was instead a very considerable accumulation of emerald xanthopterin, similar to that observed in + eyes in the same host (cf. Table 3). The SP group is much more reduced in the sed host than in the normal host. This might indicate a direct link between one of the SP components and isoxanthopterin metabolism. With regard to HB, the sed environment determines a level which is intermediate between the excess in a rosy host and the normalized concentration in +. The drop in this fraction in a sed host is probably due to HB2. It is instructive to compare Table 5 with Table 3 and to observe the parallels in the reactions of rosy and + eyes in the sed hosts.

The correlation obtained in previous experiments with rosy (Hadorn & Graf, 1958) for the adjustment in drosopterines and in isoxanthopterin had suggested a close association between the synthesis of these two pteridine classes. The present results do not support this assumption. In the hierarchy of the rosy syndrome, at all events, the two pteridine defects must occupy widely differing ranks.

C. Effect of excretory depletion in pteridines of host environment

Inherited synthetic blocks are not the only agents affecting the pteridine pattern of adult flies. The genotypes w and bw form considerable quantities of isoxanthopterin and of the SP and HB groups during pupal life (Hadorn, 1954; Hadorn & Ziegler-Günder, 1958). These pteridines are subsequently eliminated by the excretory system, especially with the meconia (Hadorn & Kürsteiner, 1955). The general correspondence between isoxanthopterin levels of hosts and transplants (cf. sections A and B) which becomes manifest in the early pupa (section A) establishes the dominance of the host in setting the rate of synthetic activity. It seemed important to find out whether pteridine retention in the transplants is also linked up with storage or depletion in the host.

The pteridine pattern was therefore examined in wild-type eyes developed in the abdomens of w hosts (Table 6). The transplants contained, on the average, two-thirds of the drosopterin quantity which is found in an eye in situ. This is as much as a transplant will usually develop in a normal host (Hadorn & Schwinck, 1956; Hadorn, 1957). Thus the red pigments behave autonomously in this experiment, as claimed by Beadle & Ephrussi (1936) from inspection of the eye-colour. We are here exclusively concerned with the other pteridines.

The relative concentrations of these substances were found to be essentially normal although the SP fraction is more abundant than in wild-type controls. Similarly, w transplants in normal hosts had followed their intrinsic excretory
pattern and only non-measurable traces of pteridines were found to be retained by them.

Table 6
The influence of white eyed hosts (w) on pteridine fractions of normal eye-transplants

DP: the mean fluorometric reading for + controls is fixed as unity. XP, SP, and HB: for each spot, the readings of these groups were expressed as fractions of DP readings.

<table>
<thead>
<tr>
<th></th>
<th>DP</th>
<th>XP</th>
<th>SP</th>
<th>HB</th>
<th>No. meas.</th>
<th>No. transl.</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ controls</td>
<td>1.00±0.02</td>
<td>1.00±0.03</td>
<td>1.00±0.04</td>
<td>1.00±0.04</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>+ eyes in w hosts</td>
<td>0.68±0.04</td>
<td>1.06±0.04</td>
<td>1.27±0.04</td>
<td>1.07±0.05</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>

It may be concluded that pteridine levels of hosts and transplants are independent during later pupal and imaginal life. The host influence via diffusible products is restricted to the phase of pteridine synthesis. Pteridine excretion in the w genotype appears to be cell-specific rather than governed by gradients pervading the whole organism.

D. Shifts in isoxanthopterin requirement within the host-transplant system

The non-autonomy of isoxanthopterin synthesis in eyes and in testes (Hadorn, Graf, & Ursprung, 1958) implies the production by the host of a prerequisite compound for this reaction. It is tempting to assume that this substance may be the enzyme xanthine-dehydrogenase (cf. Forrest, Glassman, & Mitchell, 1956), although, in this case, its penetration into the transplants would not be easy to visualize.

The main concentration of isoxanthopterin occurs in eyes and testes. A smaller quantity is present in the Malpighian tubules. These organs may be assumed to compete for the host-supplied 'agent'. It is possible to increase the demand for this substance in eye-tissues by charging a host with a maximum of eye-transplants. Hadorn (1957) has shown that a host is able to support up to eight implanted eye-disks. The drosoprotein content in each transplant of such a group was similar to that of an eye transplanted singly. The red pigments of host eyes were also undiminished.

It was decided to study the remaining pteridines in such an overloaded system. Five or six wild type eye-disks were transplanted into normal larvae. The changes in the eyes of hosts and transplants which appear in Table 7 a, b came as a surprise. A net increase in isoxanthopterin content of host eyes and transplants is obtained. The concentration of the SP + HB fraction is also higher than in the controls. Two-dimensional chromatography indicates (Table 7 b) that the SP fraction contributes largely to this effect.

It is probably wrong to attribute this excess in eye isoxanthopterin through-
out the system to a stimulated production of the ‘agent’ by the host. The key to the phenomenon is to be found in the behaviour of the host testes. Hadorn, Graf, & Ursprung (1958) observed that the isoxanthopterin content of a testis is proportional to its size. A testis which fails to establish contact with a vas deferens

**Table 7a**

The influence of increased isoxanthopterin supply on host eyes and transplants (from one-dimensional chromatograms)

5–6 wild-type eyes were transplanted into wild-type hosts.

<table>
<thead>
<tr>
<th>T6</th>
<th>T5</th>
<th>H6</th>
<th>H5</th>
<th>+ contr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IX</td>
<td>SP</td>
<td>HB</td>
<td>No. of meas.</td>
<td>No. of transpl.</td>
</tr>
<tr>
<td>----</td>
<td>----</td>
<td>----</td>
<td>-------------</td>
<td>---------------</td>
</tr>
<tr>
<td>1.51±0.08</td>
<td>1.22±0.04</td>
<td>11</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>1.34±0.07</td>
<td>1.19±0.08</td>
<td>7</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>1.24±0.09</td>
<td>1.07±0.05</td>
<td>6</td>
<td>..</td>
<td></td>
</tr>
<tr>
<td>1.19±0.05</td>
<td>1.07±0.04</td>
<td>5</td>
<td>..</td>
<td></td>
</tr>
<tr>
<td>1.00±0.03</td>
<td>1.00±0.03</td>
<td>15</td>
<td>..</td>
<td></td>
</tr>
</tbody>
</table>

**Table 7b**

The influence of increased isoxanthopterin supply on host eyes and transplants (from two-dimensional chromatograms)

For explanation cf. Table 7a. Pteridine fractions for each chromatogram are recorded in percentage of DP readings

<table>
<thead>
<tr>
<th>T6 and T5</th>
<th>H6 and H5</th>
<th>+ controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>IX</td>
<td>XP</td>
<td>SP</td>
</tr>
<tr>
<td>94.0±7.3</td>
<td>21.8±2.4</td>
<td>41.0±3.2</td>
</tr>
<tr>
<td>69.8±5.0</td>
<td>19.8±1.8</td>
<td>44.9±8.7</td>
</tr>
<tr>
<td>47.0±3.3</td>
<td>20.4±4.2</td>
<td>33.0±2.4</td>
</tr>
</tbody>
</table>

remains relatively small and rounded and accumulates only half of the isoxanthopterin quantity that is present in the normally attached and coiled organ. The testes of host males harbouring six eye-transplants exhibit invariably some slight compression. In some cases they may be severely deformed. The pteridine inventory of normally coiled and of damaged host testes is presented in Table 8. The isoxanthopterin of damaged testes is reduced. The trend in the normally coiled testes is similar.

The extent of the damage incurred by the compressed testes may be estimated from the fact that their colour was orange instead of yellow owing to the accumulation of a reddish-brown pigment. Chromatography indicates that these pig-
ments are not drosopiterines. They probably belong to the class of ommochromes. This reaction of the testes to trauma is in conformance with the findings of Ursprung, Graf, & Anders (1958) who observed ommochromes in Malpighian tubules injured by UV irradiation. In view of this serious disturbance we should refrain from basing any inferences on the abnormal pteridine ratios of the compressed testes (cf. Table 7).

**Table 8**

Influence of compression and damage on pteridine content of host testes

For explanation of experiment cf. Table 7a. IX: the mean fluorometric reading for unoperated controls is fixed as unity. XP, SP, HB: for each spot, the readings of these groups were expressed as fractions of DP readings. The mean ratios determined for unoperated controls were fixed as unity.

<table>
<thead>
<tr>
<th></th>
<th>IX</th>
<th>XP</th>
<th>SP</th>
<th>HB</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>1.00±0.03</td>
<td>1.00±0.07</td>
<td>1.00±0.04</td>
<td>1.00±0.03</td>
<td>11</td>
</tr>
<tr>
<td>Host testes slightly damaged</td>
<td>0.91±0.06</td>
<td>1.40±0.12</td>
<td>1.25±0.11</td>
<td>1.12±0.10</td>
<td>11</td>
</tr>
<tr>
<td>Host testes severely damaged</td>
<td>0.73±0.06</td>
<td>1.48±0.17</td>
<td>1.20±0.06</td>
<td>1.17±0.11</td>
<td>5</td>
</tr>
</tbody>
</table>

It is obvious, however, that these testes were unable to claim their full share of the prerequisites for isoxanthopterin synthesis. The host eyes as well as the transplants were able to profit from this fact. The advantage of transplants over host eyes in this competition must be connected with their abdominal position in the vicinity of the disturbed testes. A direct supply of HB\textsubscript{i} from testes to transplants may be involved. The increased isoxanthopterin metabolism in the eyes is associated with an increase in the SP group.

**DISCUSSION**

There are numerous inherited differences in isoxanthopterin levels of *D. melanogaster*. Males possess more of this substance than females (Hadorn & Ziegler-Günder, 1958). Major genes give rise to changes and sometimes to blocks in isoxanthopterin synthesis (Hadorn & Mitchell, 1951; Hadorn & Schwinck, 1956). Wild-type stocks exhibit much variation with regard to this character (Robertson & Forrest, 1957). It is doubtlessly dependent on a large number of genes.

Nevertheless, when two such different genotypes are combined in a transplantation experiment, the outcome appears to be uniform; the genotype of the host determines the isoxanthopterin content of the transplant. For the mutant *rosy* this conclusion was reached by Hadorn & Schwinck (1956) and by Hadorn & Graf (1958). Non-autonomy of the difference between males and females could also be demonstrated (Hadorn & Ziegler-Günder, 1958; Hadorn, Graf, & Ursprung, 1958). The present study establishes non-autonomy for the severe defect in isoxanthopterin which characterizes the mutant *sepiaoid.* In all these cases the dominance of the host in setting the rate for isoxanthopterin synthesis...
might be ascribed to a single diffusible ‘agent’ which is supplied to the transplant primordia in gene-conditioned quantities.

It could be shown that non-autonomous changes in the rate of isoxanthopterin formation are correlated with shifts in at least three other pteridine fractions. Not only the known precursor of isoxanthopterin (HB), but also the ‘xanthopterin’ and SP groups, are affected. The levels of all these pteridines are therefore host-dependent, although it may be correct to consider isoxanthopterin synthesis as the primary non-autonomous process.

Table 9

*Correlated changes negative (—) or positive (+) of pteridine groups*

The behaviour of the XP group should be interpreted with caution. It may reflect peculiarities of the *sed* genotype.

1. *Correlations with isoxanthopterin formation*

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Table</th>
<th>XP</th>
<th>SP</th>
<th>HB</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ in <em>sed</em> compared with + in +</td>
<td>3 <em>a, b</em></td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td><em>sed</em> in + &quot; &quot;</td>
<td>4</td>
<td>—</td>
<td>?</td>
<td>—</td>
</tr>
<tr>
<td><em>ry</em> in + &quot; &quot;</td>
<td>5</td>
<td>—</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Damage to testes increases IX synthesis in transplants and host eyes</td>
<td>Text-fig. 1</td>
<td>7 <em>b</em></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

2. *Correlations with drosopterin formation*

<table>
<thead>
<tr>
<th>Type of experiment</th>
<th>Table</th>
<th>XP</th>
<th>SP</th>
<th>HB</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ry</em> in <em>sed</em> compared with <em>ry</em> in <em>ry</em></td>
<td>Text-fig. 1</td>
<td>+</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 9 summarizes some of the correlations between these non-autonomous pteridines, as observed in the present experiments. It presents repeated evidence for an increase in the SP fraction with rising IX level and for an accumulation of xanthopterin when IX synthesis is inhibited. This latter phenomenon is so pronounced that it cannot be disregarded as an artefact of alkaline chromatography (Ziegler-Günder & Hadorn, 1958). However, it should be stressed that in *rosy* hosts (Hadorn & Graf, 1958) a normal transplant which is blocked in its IX production exhibits no increase in its XP fraction. This storage may be peculiar to *sed* metabolism.

A stepwise repair of the two pteridine defects in the mutant *rosy* has contributed some information on drosopterin formation. A *sed* host enables *ry* transplants to build up a normal quantity of red pigments without correcting their lack of isoxanthopterin. It appears that components of both the SP and the HB group participate in the formation of drosopterines. This experimental separation of two *rosy* phenes is not easily compatible with the concept of a single *ry* + agent, affecting DP and IX directly and jointly.
Reduction in the size of the testes due to pressure exerted by multiple eye transplants induces increased isoxanthopterin storage in both the foreign and the host eye-rudiments. We infer that under normal conditions the different organs compete for a limited supply of isoxanthopterin. In this experiment again, increased IX formation is coupled with the storage of an SP component.

The elimination of isoxanthopterin and of other pteridines in the genotype \textit{w} (Hadorn, 1954) is a cell-specific process. It is not regulated by the composition of the haemolymph or by the activity of the Malpighian tubules. A \textit{w} eye excretes its pteridines in a wild-type environment and a normal eye is able to maintain its fluorescent substances in a \textit{w} host. Thus, pteridine retention, in contrast to pteridine synthesis, involves no communication between host and transplant.

**SUMMARY**

1. The isoxanthopterin deficiency of the mutant \textit{sepiaoid} is a non-autonomous character.
2. Non-autonomous changes in the level of isoxanthopterin are correlated with changes in three other pteridine fractions.
3. A \textit{sepiaoid} host is able to normalize the red pigments in implanted eyes of the mutant \textit{rosy}, but does not repair its isoxanthopterin defect. The reaction of other pteridines to drosopterin formation can thus be separately observed.
4. While isoxanthopterin synthesis may generally be host dependent, its retention in the imago is autonomous. Wild-type eyes maintain their pteridine levels in a \textit{white} host that excretes its own fluorescent materials.
5. Compression of the testis by numerous eye transplants diminishes its isoxanthopterin storage. The surplus of diffusible precursors leads to increased deposition in host eyes and eye-transplants.

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**REFERENCES**


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