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[15] This has been observed by Bauer (*Zeits. f. Zellf.*, **23**, 280, 1935), in *Chironomus* and in *Drosophila* (*Proc. Nat. Acad. Sci.*, **22**, 216, 1936) and it appears also to be true in *Sciara*, but Yasui (*Cytologia*, **6**, 330, 1935) and Tiniakov[4] maintain that the salivary chromosomes in *Drosophila* are flat tapes, and Koltzoff,[12] Heitz (*Biol. Zbl.*, **54**, 588, 1934) and Muller[10] that they are hollow cylinders.
[16] In the definitive salivary chromosomes of *Sciara* the visible striations and granules in some regions do appear to be approximately the same size and distance apart as the original chromomeric threads, but in many other regions they are either more or less numerous.

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DEVELOPMENT OF EYE COLORS IN DROSOPHILA: RELATION BETWEEN PIGMENTATION AND RELEASE OF THE DIFFUSIBLE SUBSTANCES

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1. *Introduction*.—Eye buds of the mutants vermilion (*v*) or cinnabar (*cn*) of *Drosophila melanogaster*, implanted into larvae of the mutant white (*w*) develop into eyes which are wild type in color. It has been previously shown that a similar modification of *v* and *cn* eyes implanted into wild type is due to the presence of two diffusible substances (the v^+ and cn^+ substances) in the lymph of the wild type hosts.[2] It must be concluded then that the same substances are present in the *w* mutant and that the partial or total absence of pigment in the mutants of the *w* series is due to the disturbance of a mechanism other than that which leads to the formation of diffusible substances required in the development of the wild type eye color.

It has also been shown[2,3] that eye implants of various mutants can produce and sometimes release the diffusible substances; this can be shown by implantation of the test eyes into larvae sensitive to one of the substances; if, for example, eye buds that release the v^+ substance are implanted into apricot-vermilion ($w^a v$) hosts, there is a modification of the host eyes toward the apricot (w^a) phenotype.

Experiments of this type have shown that the amounts of substances released by the different mutant eyes vary a great deal with the nature of the mutant considered; and there appeared to be a relation between the amount of substance released and the degree of pigmentation of the implant itself.

The primary purpose of the experiments described below has been to establish in a direct way this relation between the release by a given eye of the two substances mentioned and its actual pigmentation. The different w allelomorphs which form a series of increasing intensities of pigmentation, appeared to fit our purposes particularly well and have, therefore, been used in the present experiments.

TABLE 1

RELEASE OF THE v^+ SUBSTANCE BY THE EYES OF FOUR w-ALLELOMORPHS

IMPLANT	HOST	PHENOTYPE OF HOST	NUMBER OF INDIVIDUALS
w	$w^a v$	w^a	8
w^h	"	Slightly lighter than w^a	10
w^{ch}	"	Intermediate between w^a v and w^a	7
w^{331}	"	w^h v	11

TABLE 2

RELEASE OF THE cn^+ SUBSTANCE BY THE EYES OF FOUR w-ALLELOMORPHS

IMPLANTS	HOST	PHENOTYPE OF THE w^a; cn IMPLANT	NUMBER OF INDIVIDUALS
w and w^a;cn	v;cn	w^a	9
w^h and w^a;cn	"	w^a	6
w^{ch} and w^a;cn	"	Intermed. between w^a;cn and w^a	3
w^{331} and w^a;cn	"	w^a;cn	8

2. *Release of the v^+ substance.*—The release of v^+ substance by four members of the w series (w, honey (w^h), cherry (w^{ch}) and white-331 (w^{331}) used as implants, has been evaluated by their effect on w^a v hosts, sensitive to the v^+ substance. The results of this experiment are shown in table 1.

These data suggest that the release of v^+ substance is inversly proportional to the intensity of pigmentation of the eye used as the source of the substance.

3. *Release of the cn^+ substance.* The release of the cn^+ substance by the same w allelomorphs has been measured by simultaneous implantation of these test eyes and of w^a;cn eyes (sensitive to the cn^+ substance) into

v;cn hosts, known to be lacking both the v^+ and the cn^+ substances. The results of these experiments are given in table 2.

These data indicate an inverse proportionality between the release of the cn^+ substance and the pigmentation of the eye used as the source of the cn^+ substance.

It should be pointed out that in the experiments cited, the $w^a;cn$ eye itself constitutes a source of a small amount of v^+ substance[4,5] which can be converted to cn^+ substance by the other implant. However, it is obvious that the amount of this v^+ substance is small and constant enough, so that it does not materially affect the result.

4. *Conversion of the v^+ substance to the cn^+ substance.*—The $w^a;cn$ eyes have been implanted into a series of five double recessives of *v* with an allelomorph of *w*. This series is: white-vermilion (*w v*), apricot-vermilion ($w^a\ v$), blood-vermilion ($w^{bl}v$), coral-vermilion ($w^{co}v$) and white-331-vermilion ($w^{331}\ v$). These five double recessives also show an increasing intensity of pigmentation, the difference between $w^{co}\ v$ and $w^{331}\ v$ being

TABLE 3

CONVERSION OF THE v^+ SUBSTANCE TO cn^+ SUBSTANCE

IMPLANT	HOST	PHENOTYPE OF IMPLANT	NUMBER OF INDIVIDUALS
$w^a;cn$	$w\ v$	w^a	1
"	$w^a\ v$	w^a	7
"	$w^{bl}\ v$	Intermed. between $w^a;cn$ and w^a	6
"	$w^{co}\ v$	"	9
"	$w^{331}\ v$	$w^a;cn$	6

extremely small at 25°C. Because homozygous for *v*, they are unable to produce any of the two substances considered, but they can convert the small amount of v^+ substance supplied by the $w^a;cn$ implant to cn^+ substance. The degree of modification of the $w^a;cn$ implant toward w^a serves to evaluate the relative amount of substance converted.

It can be seen from table 3 that the more pigment contained in the host eyes, the less the effect on the implant. In other words, the host received a constant amount of v^+ substance from the implant and converted a fraction of it to cn^+ substance; this fraction appears to be inversely proportional to the intensity of pigmentation of the host eyes.

5. *Release of the cn^+ substance in the Presence of an Excess of v^+ substance.*—It is interesting to compare the experiments described in paragraph 3 with an experiment involving the implantation of eyes of the *w* series into $w^a;cn$ hosts. The eyes serving as the source of the substance and those serving as receptors are the same in the two experiments. The difference between the two experiments lies in the fact that in the experiments reported above the eye serving as the source of the cn^+ substance

(w allel) received from the $w^a;cn$ implant only a very small amount of the v^+ substance, while in the experiment to be reported below (implantation of eyes of the w series into $w^a;cn$) the eye serving as source of cn^+ substance (the same w allel) is placed in a medium containing an excess of v^+ substance. Table 4 shows that the extreme effect (complete change to w^a), which has previously been produced only by w and w^h implants, is obtained now by implantation of w, w^h and w^{ch}; and that w^{331} produces here a clear effect, while it produced no effect in the experiments described in paragraph 3.

It is clear, then, that the ability to convert the v^+ substance into the cn^+ substance, as measured by the release of the latter, also depends on the

TABLE 4

RELEASE OF THE cn^+ SUBSTANCE IN THE PRESENCE OF AN EXCESS OF THE v^+ SUBSTANCE

IMPLANT	HOST	PHENOTYPE OF HOST	NUMBER OF INDIVIDUALS
w	$w^a;cn$	w^a	11
w^h	"	"	9
w^{ch}	"	"	11
w^{331}	"	Intermed. between $w^a;cn$ and w^a	10

TABLE 5

RELEASE OF THE v^+ SUBSTANCE BY EYES HOMOZYGOUS FOR EITHER $bw;cd$ OR $pn^2;cd$ COMPARED WITH THAT OF bw, pn^2 AND cd EYES

IMPLANT	HOST	PHENOTYPE OF HOST	NUMBER OF INDIVIDUALS
bw	$w^a\ v$	$w^a\ v$	*
cd	"	"	*
pn^2	"	"	*
$bw;cd$	"	Intermed. between $w^a\ v$ and w^a	13
$pn^2;cd$	"	"	7

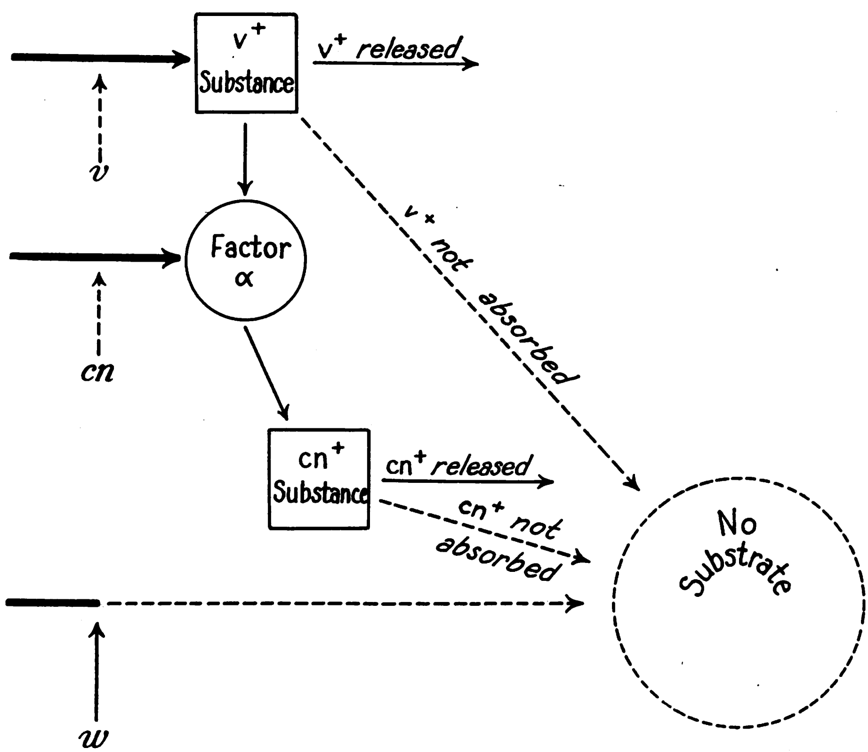
* Data of Ephrussi and Beadle.[3]

actual pigmentation of the eye. In paragraph 4 the conversion was brought about by a v eye, while here a v^+ eye causes the same transformation. This shows that the relation which we have established holds in the two cases or that, in other words, an eye which itself produces the two substances can nevertheless transform a certain amount of v^+ substance supplied from another source.

6. *Does the Relation between Release of the Diffusible Substances and Pigmentation Apply to Other Cases than the w Mutants?*—Before considering the observed relation between intensity of pigmentation and release of substances as proved, we had to eliminate a possible objection. It had to be demonstrated that the release of substances is not associated in a purely fortuitous manner with the quantity of pigment shown in the eyes of the

different *w* allelomorphs. To do this the release of v^+ substance by the eyes of two double-recessives has been examined. We have chosen the mutants brown (*bw*), cardinal (*cd*) and prune-2(pn^2) because the double recessives *bw;cd* and $pn^2;cd$ are very light as compared with the simple mutants and because none of those simple mutants release the v^+ substance when tested by implanation into w^a *v* flies.[3]

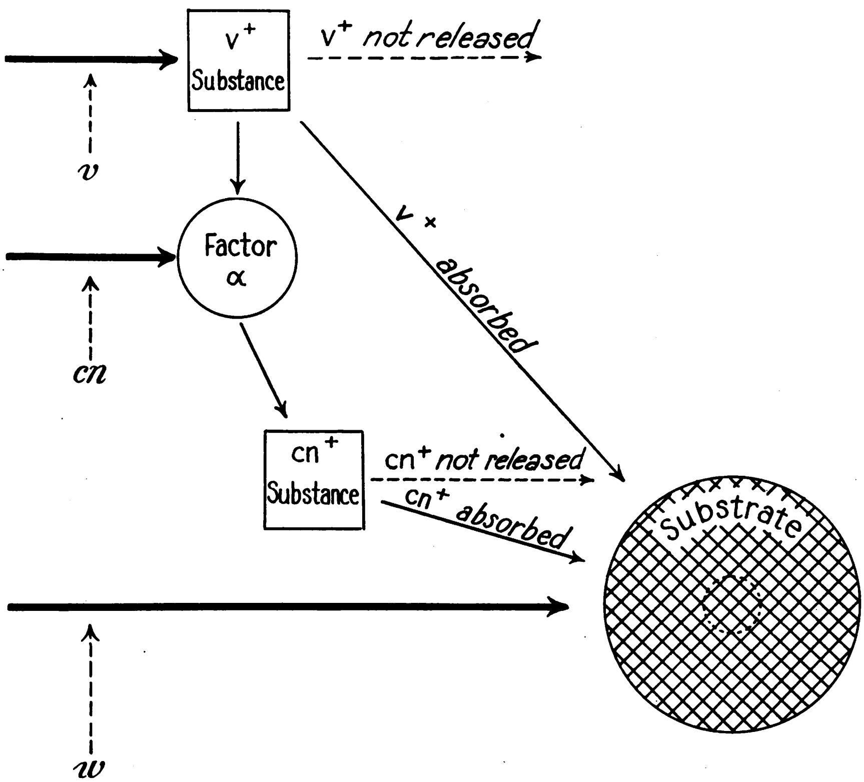
The results of these experiments are shown in table 5, from which one can see that both double recessives do release the v^+ substance. This is in



agreement with the assumption that there is a causal relationship between the release of the substances by a given eye and its actual pigmentation.

7. *Discussion.*—As suggested by Ephrussi and Beadle,[6] the amount of substance released by a given eye can be considered as equal to the difference between the amount produced and the amount used by the eye itself. Experiments constituting additional evidence which gives support to this view are published elsewhere[7] and, therefore, are not considered here. It will be mentioned only that these experiments have shown that an eye producing v^+ substance will not release the substance as long as its own

requirements are not satisfied. The same rule applies to the release of the cn^+ substance as well as to the transformation of the v^+ substance into the cn^+ substance when this transformation is evaluated by the release of the cn^+ substance.



FIGURES 1 AND 2

Schematic representation of the relation between release of diffusible substances and eye-pigmentation. Vertical arrows above symbols indicate points at which the independent formation of the v^+ substance, of the factor α and of the substrate, may be interrupted by the intervention of the mutant genes (solid arrows indicate reaction is taking place). Further explanation in text.

On the basis of these facts, taken together with the results of the experiments reported in the present paper, the following scheme of action of the v^+ and cn^+ substances in the process of pigment formation is proposed.

It will be recalled that Beadle and Ephrussi[2] have advanced the following scheme for the formation of the v^+ and the cn^+ substances:

$$\longrightarrow v^+ \longrightarrow cn^+$$

Figures 1 and 2 show the way in which we propose to complete this scheme. It is known that the v^+ substance is necessary for the formation of the cn^+ substance. We assume that the formation of the cn^+ substance results from the interaction of the v^+ substance with what we call "factor α." As is indicated by the vertical dotted arrows, the formation of the v^+ substance and that of the "factor α" (and, therefore, of the cn^+ substance) can be interrupted independently of each other by the intervention of the mutant genes *v* and *cn*. In order to account for the relation between the intensity of pigmentation and the release of substances, we postulate another factor: the "substrate," which we consider is a precursor of the pigment and which, therefore, is variable. The formation of the substrate is more or less inhibited by the intervention of the *w* allelomorphs. The transformation produced by the v^+ and cn^+ substances would be brought about by their action on the substrate.

In the case of eyes with little or no substrate (case of the mutant *w* which blocks the formation of pigment) the v^+ and cn^+ substances would not be used or would be used only in small amounts and, therefore, could be released (Fig. 1).

In the case of eyes rich in substrate (heavily pigmented eyes) the diffusible substance would be entirely consumed by the substrate and, therefore, not released (Fig. 2).

All intermediates can be easily imagined between these two extreme cases.

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