the same proportionate degree as in the females. The length of life was not significantly influenced in either direction. There were no "premature" deaths among the 26 male rats used in these experiments. Nor have we observed in any of these males the symptoms of excessive nervousness which occasionally appeared among the females as previously described.

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ON THE PROTEINS OF a^+a^+ AND aa EPHESTIA

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It has been shown previously that lack or reduction of pigment in a number of organs in Ephestia homozygous for the gene a is due to lack of kynurenin, a precursor of the pigment.[1] Kynurenin is formed from tryptophane in the metabolism of a^+-animals. In aa moths the tryptophane content of the proteins is higher than in the wild type.[2] Since no concomitant increase in protein nitrogen was found, it was concluded that either a new type of protein is formed under the influence of the gene a, or that proteins rich in tryptophane replaced others poor in tryptophane. In either case, the protein make-up of the cell would be changed.

Since proteins perform important functions in the cell, it should be expected that other characters of the cell are also changed. Actually it has been found that fat content,[2, 3] viability,[4] speed of development[4] and possibly O_2 consumption of homogenates[5] are reduced in aa animals. It has been concluded that as a result of the mutation $a^+ \rightarrow a$ the biochemical make-up of the cell is changed in several respects.

The characters influenced by the genes a^+ and a are summarized in table 1. Included in this table are the effects of a third allele a^k as far as they are known. Besides differing in a number of pigmentation characters, a^+ and a differ from each other in chemical composition (kynurenin content, tryptophane content, fat content), and in some general biological characteristics such as viability and speed of development. The gene a^k appears intermediate between a^+ and a, as far as pigmentation characters are concerned. The same appears to be true for kynurenin content, since a^ka^k mothers were able to pass on a substance capable of darkening larval ocelli to their a^ka offspring, and since the same maternal effect was observed in aa

offspring from aa mothers into which degenerating $a^k a^k$ ovaries had been grafted.[6] The speed of development, on the other hand, is still more re- duced in $a^k a^k$ than in aa. With regard to pigmentation characters and kynurenin formation, the series of genes would be $a^+ > a^k > a$, while with regard to speed of development they would be arranged in the order $a^+ > a > a^k$. Similar differences in serial arrangement in multiple alleles accord- ing to the characters observed have been repeatedly found. The remaining characters have not been investigated in $a^k a^k$.

It appears difficult to decide in which physiological order the different characters observed should be arranged. It was proved by injection ex- periments[1] that the pigment reduction in aa is due to lack of kynurenin. A

TABLE 1

PLEIOTROPIC EFFECTS OF THE GENES a^+, a^k AND a

A. Pigmentation characters	a^+a^+	$a^k a^k$	aa
1. Eyes	Black	Brown	Red
2. Brain	Brown	....	Light pink
3. Testes	Pigmented	Weakly pigmented— colorless	Colorless
4. Larval ocelli	Strong pigmentation	Intermediate pigmentation	Weak pigmentation
5. Larval hypodermis	Pigmented	Colorless	Colorless
6. Proteins	Pink	....	White
B. Chemical constitution			
7. Kynurenin	Present	Reduced in amount	Strongly reduced or absent
8. Protein tryptophane	....	?	Increased
9. Ether-extractable substances	....	?	Reduced
C. General biological characteristics			
10. Viability		Reduced	Reduced
11. Speed of development		Strongly reduced	Reduced
12. O₂ consumption in homogenates		?	Reduced ?

block of the oxidation of tryptophane to kynurenin may be considered to be the primary effect of the gene a. The non-oxidized tryptophane would then be stored secondarily in part in the proteins which are consequently quali- tatively changed. The reduction in fat content, in viability and in speed of development may be assumed to be a consequence of the altered protein constitution. In $a^k a^k$, the amount of kynurenin seems to be smaller than in a^+-, but larger than in aa. The proteins of $a^k a^k$ may therefore be expected to be intermediate in tryptophane content. Unfortunately, this assumption could not be investigated. It can be imagined, however, that the resulting $a^k a^k$ proteins have a more profound action on speed of development than

the *aa* proteins. Considerations of this kind may explain the frequent occurrence of non-seriability found in multiple alleles.

An alternative assumption would be that the formation of certain proteins rich in tryptophane is the primary step caused by the gene *a*. In this case tryptophane bound up in the proteins would not be available for kynurenin formation. Again the change in fat content, etc., may be another consequence of the changed protein constitution. If the difference in protein constitution were the primary effect of the gene *a*, causing less tryptophane to be available for the formation of kynurenin, it might be expected that *aa* proteins might release tryptophane at a slower rate than a^+a^+ proteins under the influence of proteolytic enzymes. With this possibility in mind, autolysis was studied in a^+a^+ and *aa* homogenates. In the experiments, fully grown a^+a^+ and *aa* larvae were used. The *aa* strain had been crossed out previous to the experiment for 8 and 9 consecutive generations to the a^+a^+ strain used, so that the alleles were compared on a reasonably isogenic background. 2.5–3.0 g. larval material were finely ground in homogenizers according to Potter and Elvehjem in 3 ml. Ringer solution isotonic for Ephestia and buffered with 0.02 M phosphate buffer to pH 6.8. The larvae were homogenized at 0°C. for 10 minutes. In the homogenates, total tryptophane was determined according to the method of Sullivan and Hess,[7] and total nitrogen by a micro Kjeldahl procedure. One ml. of the homogenate was pipetted into each one of 5 small test tubes and incubated in a water bath at 31°C. The homogenate was examined for non-protein nitrogen and for non-protein tryptophane at 0, 1, 2, 4 and 6 hours after the start of the experiment. 0.1 ml. of 3 M trichloroacetic acid was added to each tube, and the precipitate filtered off and washed with 0.4 ml. H_2O. In the filtrate, tryptophane and nitrogen were determined. Seven paired experiments with both a^+a^+ and *aa* homogenates and one experiment using a^+a^+ material only were run.

At time 0, there are 8 measurements of tryptophane and nitrogen for a^+a^+, and 7 for *aa* which can be used to indicate the tryptophane content of a^+a^+ and *aa* larval material. The data are given in tables 2a and b. In

TABLE 2a

TRYPTOPHANE AND NITROGEN CONTENT IN HOMOGENATES OF ISOGENIC a^+a^+ AND *aa*
EPHESTIA IN MG./G. WET WEIGHT

STRAIN	TOTAL N	NON-PROTEIN N	TOTAL TRYPTOPHANE	NON-PROTEIN TRYPTOPHANE
a^+a^+	13.8	1.49	1.22	0.04
aa	14.5	1.43	1.42	0.10

table 2a, the values for protein and non-protein nitrogen and tryptophane are given, expressed in relation to wet weight. Qualitatively, the data indicate an increase in total and non-protein tryptophane in *aa* as compared

to a^+a^+ without a concomitant increase in nitrogen. For a quantitative analysis, these data are unsuited because of the variability of both nitrogen and tryptophane in relation to wet weight. Quantitative data for tryptophane in relation to nitrogen content are given in table 2b.

TABLE 2b

RATIOS OF TRYPTOPHANE AND NITROGEN IN THE TOTAL AND NON-PROTEIN FRACTION OF a^+a^+ AND aa EPHESTIA HOMOGENATES

	a^+a^+	aa	t	$P_{df=13}$
$\dfrac{\text{Total tryptophane}}{\text{Total N}}$	0.088	0.098	2.251	< 0.05
$\dfrac{\text{Non-protein tryptophane}}{\text{Non-protein N}}$	0.027	0.070	3.241	< 0.01
$\dfrac{\text{Non-protein N}}{\text{Total N}}$	0.108	0.099	0.068	> 0.5
$\dfrac{\text{Non-protein tryptophane}}{\text{Total tryptophane}}$	0.033	0.070	3.315	< 0.01

The ratio total tryptophane/total nitrogen is increased in aa as compared a^+a^+. The difference is slightly below the 0.05 level of significance, i.e., just on the borderline of statistical significance. It appears, however, that this difference is real, in view of previously published results,[7] in which it has been found both for aa imagoes and aa larvae that the tryptophane content as related to dry weight is increased in comparison with a^+a^+ animals. Furthermore, the second line of table 2b shows that the non-protein tryptophane expressed in terms of non-protein nitrogen is higher in aa than a^+a^+ homogenates. This difference is well below the 0.01 level of significance. It should be pointed out that in the data involving non-protein tryptophane the level of significance calculated is probably too high, since 2 of the 8 values for a^+a^+ were given as 0, while they may have contained amounts of tryptophane too small to be measured. On the other hand, differences in the same direction and of the same order of magnitude have been found previously for aa and a^+a^+ imagoes, so that the conclusion appears justified that in aa material the amount of non-protein tryptophane is increased. No separate values for protein tryptophane are available in the present series of experiments. The tryptophane values in table 2a, columns 3 and 4, indicate, however, that the absolute amounts of non-protein tryptophane (0.04 and 0.10 mg./g. wet weight) are too small to account for the whole difference in total tryptophane content between a^+a^+ and aa material, 0.16 mg./g. wet weight. It must be concluded that part of the increased tryptophane content of aa is stored in the proteins, in agreement with earlier direct findings in imagoes. On the other hand, the ratio non-protein tryptophane/total tryptophane is almost twice as large
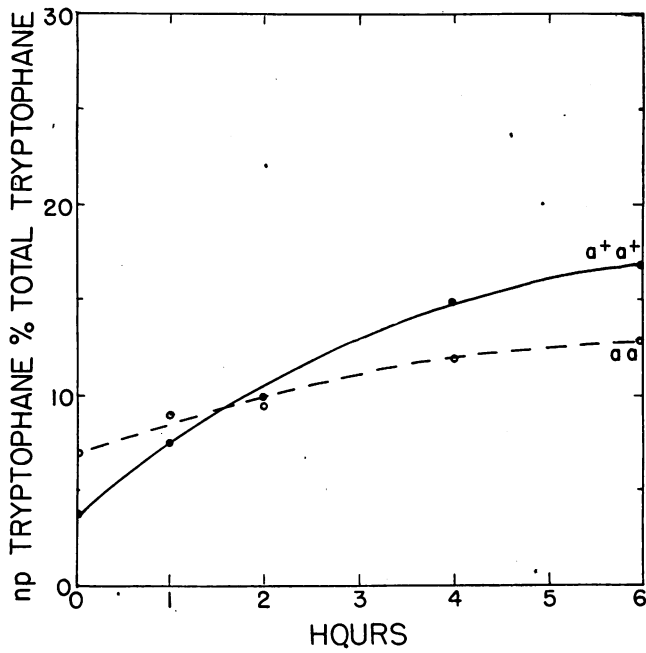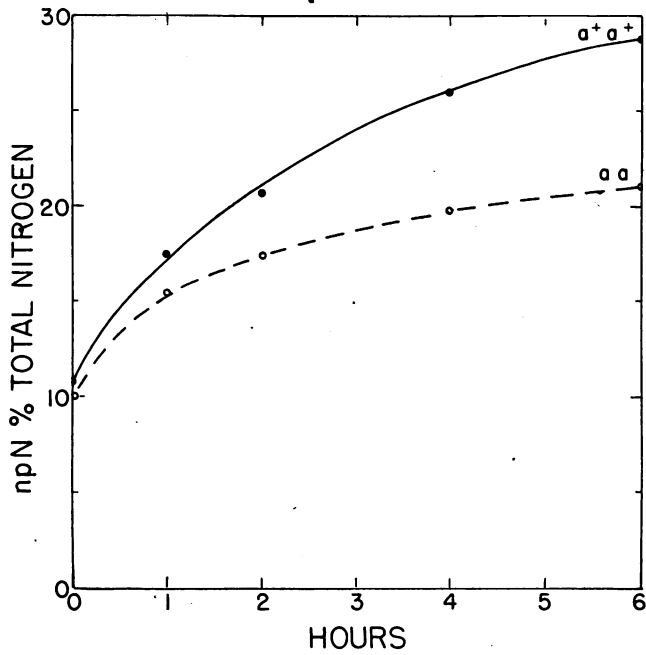
FIGURE 1

Rate of autolysis of homogenates from a^+a^+ and aa Ephestia: (*a*) release of **non-protein nitrogen**; (*b*) release of non-protein tryptophane.

in aa than in a^+a^+ larvae; a higher proportion of the tryptophane is found in the non-protein fraction in aa than in a^+a^+ material.

The results of the autolysis experiments are recorded in figure 1. In these curves non-protein nitrogen and non-protein tryptophane are expressed as per cent of the total nitrogen and total tryptophane, respectively. The points are the means of the different determinations. It should be emphasized that while the curves obtained in the individual experiments vary considerably, the relative rate of release of nitrogen and tryptophane in the two strains is consistent in all paired experiments.

The rate of autolysis appears to be very high, compared with other autolysis experiments. This may be due to the fact that old larvae nearing pupation were used in the experiments. It must be assumed that potent proteolytic enzymes are active during the breakdown of larval tissues at metamorphosis, and these enzymes may start to be produced in fully grown larvae.

There are decided differences between the rates of release of both non-protein nitrogen and non-protein tryptophane in the two strains. Both substances increase more rapidly in a^+a^+ than in aa material. The amounts of non-protein nitrogen in the two strains are originally identical. After 6 hours, the non-protein nitrogen has increased by 17.8% of the total nitrogen in a^+a^+ material while in aa homogenate it increased only by 11% of the total nitrogen. The percentage of non-protein tryptophane is originally higher in aa than a^+a^+ material, 7.0% as against 3.3% of the total tryptophane. The rate of increase in non-protein tryptophane under the influence of autolytic enzymes is again slower in aa than in a^+a^+ material, increasing in 6 hours by 6.75% in aa as against 13.5% of the total tryptophane in a^+a^+ homogenate.

The difference in the rate of autolysis may be due either to differences in the structure of the proteins or in the activity of the enzymes concerned. In either case, it would agree with the previous observation that the protein make-up of the cell is changed under the influence of the gene a. If the differences in rate of autolysis are due to a different resistance of the proteins to the same enzyme system, the possibility would not be excluded that the primary action of the gene a is the formation of different proteins richer in tryptophane and more resistant to autolysis. Both these facts would tend to reduce the amount of free tryptophane available for kynurenin formation. However, the fact that the relative amount of non-protein tryptophane is found to be increased in aa animals would tend to favor the alternative that the primary effect of the gene a is a block of the oxidation of tryptophane to kynurenin.

If tryptophane were bound in different types of linkage in a^+a^+ and aa proteins, it might possibly be released during autolysis at different rates in relation to nitrogen. Material concerning this question is given in table 3
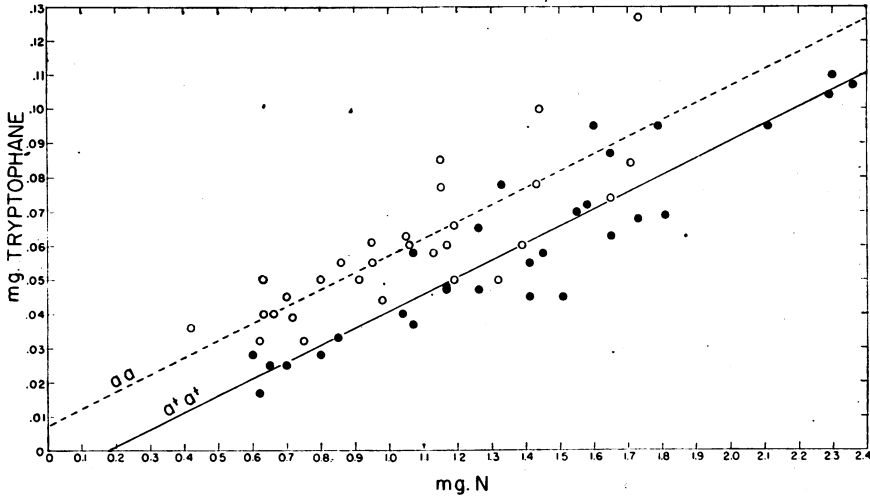
FIGURE 2

Correlation between non-protein nitrogen and non-protein tryptophane: (a) in a^+a^+ homogenates; (b) in aa homogenates. The lines represent the regression equations.

and figure 2. In figure 2, the corresponding tryptophane and nitrogen values for the same samples are plotted in a scatter diagram. If tryptophane were released at a faster rate, relative to nitrogen, in one strain than in the other, the slopes of the two curves would be different. Actually the slopes for the two strains are almost identical, the linear regression coefficients being 0.0496 and 0.0497 mg. tryptophane per mg. N, respectively. The closeness of the association of the different values found with the linear regression line and consequently the variability of the values is indicated by the correlation coefficient. The last values on table 3 indicate that the correlation coefficients for the two strains are not significantly different. All these findings give no reason to suspect that tryptophane is bound in a different way in aa than in a^+a^+ proteins.

TABLE 3

RELATION BETWEEN NON-PROTEIN NITROGEN AND NON-PROTEIN TRYPTOPHANE IN THE COURSE OF AUTOLYSIS IN a^+a^+ AND aa EPHESTIA HOMOGENATES

STRAIN	NO. OF DETERMINATIONS	REGRESSION COEFFICIENTS MG. TRYPTOPHANE/MG. N	CORRELATION COEFFICIENT
a^+a^+	30	0.0497	0.923 ± 0.027
aa	29	0.0496	0.817 ± 0.062

In view of the great number of physiological differences found between a^+a^+ and aa animals the question appears legitimate whether physiological differences of the same order of magnitude may be associated with other genes. While no direct evidence on this point is available the frequent

occurrence of characteristic effects of genes on viability[8] and on shape of the spermatheca[9] in Drosophila seem to suggest that this is the case.

Summary.—The tryptophane content of *aa* Ephestia larvae is higher than that of a^+a^+ Ephestia larvae in the non-protein fraction and probably also in the protein fraction. In homogenates of isogenic a^+a^+ and *aa* homogenates, autolysis proceeds at a faster rate in a^+a^+ than in *aa* material. Formation under the influence of the gene *a* of a protein richer in tryptophane and more resistant to proteolytic enzymes would constitute a conceivable mechanism for inhibition of kynurenin formation in *aa* animals.

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THE RAYLEIGH-RITZ AND THE WEINSTEIN METHODS FOR APPROXIMATION OF EIGENVALUES. II. DIFFERENTIAL OPERATORS

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1. In the present paper we are going to apply the results of our first paper[1] to eigenvalue problems for differential operators. In general, the problems will be of the following type. Given two linear (ordinary or partial) differential operators A and B, A of higher degree than B, the operators being defined for functions in some domain D, we consider the equation

$$Au = \theta Bu, \qquad \theta \text{ a constant parameter.} \qquad (1)$$

We want then to find functions satisfying in the domain D the equation (1) and on the boundary C of D some homogeneous boundary conditions which will be denoted by (B).