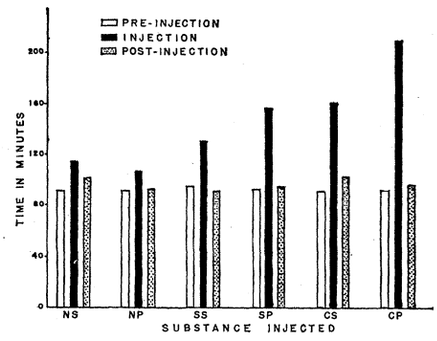

Fig. 1. Mean total time in minutes to complete the task on the day of injection of blood substances, and on the day before and after injection. Abbreviations: *NS* normal serum; *NP*, normal plasma; *SS*, stressed normal serum; *SP*, stressed normal plasma; *CS*, catatonic serum; *CP*, catatonic plasma.

mental treatments (donor category) for total number of reinforcements or for any response-duration measure. Total time taken to complete the 300-trial session, however, varied strikingly as a function of experimental treatment (see Fig. 1). Injections of serum or plasma from catatonics significantly prolonged total session time as compared with serum or plasma from normals ($p < .01$). Serum and plasma from stressed normal donors also significantly prolonged session duration ($p < .05$). Differences between the effects of blood samples from stressed normals and catatonics were nonsignificant. It is of interest, however, that three of the 12 samples from catatonics did not increase session duration at all, while two samples from catatonics produced an effect well beyond the range found for samples from stressed normals. In these two instances the monkeys required over 5 hours to complete 300 trials. The average daily time required to complete the task was 90 minutes.

The observed prolongation of session time found after injection of most samples from stressed normals and catatonics correlated with periods of apparent motor arrest. Animals would squat in fixed positions, often facing the discriminative stimulus panel but failing to respond to it. A number of other investigators have noticed a lack of responsiveness in animals injected with substances from schizophrenics. Workers utilizing the rope-climbing task described earlier, however, have not included a measure of response latency and have applied aversive stimulation to force a response. Our data indicate that the initiation of a response can be the most severely affected dimension of behavior, and that it is a pertinent variable.

The results suggest two alternate hypotheses. First, that all disruptive effects measured are allied to general stress physiology, and differences in degree of disruption correlate with the degree or stage of the stress process. Research with stressed primate donors is being instigated to provide relevant data.

An alternate hypothesis is that two separate factors are responsible for disruptive effects: one, a general factor related to stress physiology; the other, a factor specific to a limited percentage of schizophrenic cases with a common etiology (potentially represented by the two donors whose blood samples produced extreme disruption). The probability is high that a number of discrete and qualitatively different mechanisms underlie the common symptomatology of many psychiatric diagnostic categories. The dearth of biochemical breakthroughs in this field may well relate to an underlying heterogeneity among the cases grouped for study. Behavioral bioassay techniques such as the one utilized in the present study have potential value as selectors of a clinical subgroup worthy of intense biochemical study.

Finally, the data suggested the necessity for control or measurement of the stress variable in any assessment of the disruptive effects of body fluids from psychotics. The question of the specificity to psychosis of the toxic factor or factors implicated in previous studies should be more thoroughly investigated (*6*).

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6. Supported by research grants MH-1951 and MH-5822 from the National Institute of Mental Health, Public Health Service.
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24 December 1962

Beta-Alanine Utilization of Ebony and Non-ebony Drosophila melanogaster

Abstract. *Carbon-14–labeled β-alanine was injected into newly formed* Drosophila melanogaster *female pupae. Homozygous ebony deposited less* C^{14} *in pupal sheaths, deposited more* C^{14} *in adult body extracts and wings, and decarboxylated and oxidized β-alanine to excrete* $C^{14}O_2$ *faster than did non-ebony homozygotes. Heterozygotes were intermediate in all these activities.*

Early studies by L'Heritier, Neefs, and Teissier (*1*) and others indicate that the population of ebony *Drosophila melanogaster,* in competition with the non-ebony, stabilizes at a low frequency after an initially high frequency. These results are best explained in terms of low fitness of the ebony homozygote, which lowers the frequency of that genotype, and superior fitness of the heterozygote over either homozygote to prevent ebony from showing complete negative selection from the population. Elens (*2*) and Jacobs (*3*) have shown that heterozygous males are more vigorous in mating than homozygous males, especially homozygous ebony. This study was conducted to discover metabolic differences among these genotypes.

In preliminary work, samples of 16 different amino acids (C^{14} labeled) were injected into newly formed female pupae of the three genotypes, and C^{14} activity counts of pupal sheaths, wings, and body extracts as well as excreted CO_2 were made. Of the injected amino acids, only β-alanine showed appreciable differences among the genotypes. More extensive studies of this amino acid were then made and are reported here.

The flies used were ebony and a light tan wild type; all were collected at Beaufort, North Carolina (*4*). They were cultured; crosses were repeatedly made between them, and offspring were selected for homozygosity according to a previously described method (*3*). By means of an automatic micropipette, β-alanine-1-C^{14} and -2-C^{14} (100 μc, 1 mc/mmole of saturated aqueous solution at room temperature) were injected into females when they had become immobile for entering the pupal stage. Each female of each genotype was injected with about 0.005 μl in the dorsal blood sinus just posterior to the heart. In an attempt to treat the

females of each genotype the same, injections were made in a rotating fashion; only a few seconds was allowed to elapse between injection of a female of one genotype and a female of another genotype. The injected pupae were placed on a glass plate which was then inverted over a radioactive counter sampling pan into which two drops of 2 percent aqueous $Ba(OH)_2$ had been placed. These were left in a precision incubator at 25°C for various intervals. The sample pan with accumulated $C^{14}O_2$ was then removed and counted with a Geiger-Müller gas flow counter. After the last CO_2 sampling, the pupae were placed on the wall of a vial (25 by 95 mm) near the cotton plug and replaced in the incubator for emergence.

When the flies emerged in the vials, they were aged for 1 day; then the wings were cut off from a sample of the flies and laid closely together on the adhesive side of cellulose tape which was then fastened to a sample pan for counting of radioactivity. Entire pupal sheaths from which the flies had emerged were similarly counted. The heads were then removed for extraction of eye pigments; the headless and wingless bodies were boiled in water for 1 minute to inhibit enzyme action, homogenized in 70-percent ethanol, and centrifuged at 25,000*g* for 10 minutes. The supernatant was filtered through Whatman No. 1 paper into sample pans for counting.

Ebony homozygotes (e/e) injected with the carboxyl-labeled sample (β-alanine-1-C^{14}) excreted $C^{14}O_2$ at a higher rate than did non-ebony homozygotes (+/+), while heterozygotes (+/e) were intermediate (Fig. 1). This figure represents a set of four pupae of each genotype. The total number of sets injected with β-alanine-1-C^{14} and similarly studied were: 50 pupae of each genotype (one set), 20 pupae of each genotype (two sets), four pupae of each genotype (three sets) and one pupae of each genotype (ten sets). All these sets except one single-pupa set were consistent in showing e/e greater than +/e greater than +/+ in rate of excretion of CO_2 labeled with C^{14}. The greater degree of fluctuation in rate of C^{14}-labeled CO_2 excretion for e/e as compared with the others was also consistently observed.

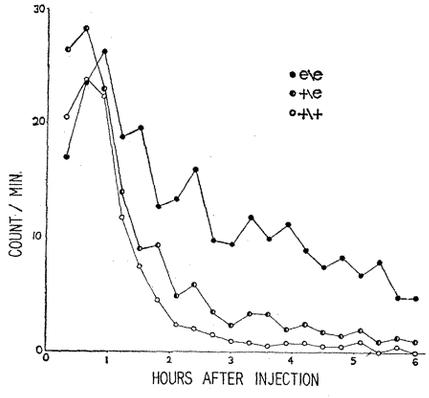

Fig. 1. Radioactivity (count/min per pupa) of C^{14}-labeled CO_2 excreted by *Drosophila melanogaster* females injected, as newly formed pupae, with β-alanine-1-C^{14}. (Collections were made continuously for intervals of 18 minutes after injection.)

When β-alanine-2-C^{14} was injected, and $C^{14}O_2$ excretion was measured, the results were similar to those observed with β-alanine-1-C^{14}. This analysis is based on four sets of 15 pupae per genotype. All four sets showed essentially the same picture. There was not, however, as sudden an increase in excretion rate immediately after injection with β-alanine-2-C^{14} as had been observed when the -1-C^{14} had been injected. Rather, there was a gradual increase for about 2 hours after injection, and then a dropping off in rate was observed. Some of the sets injected with both β-alanines were sampled for $C^{14}O_2$ excretion on days following the day of injection. In these sets, the activity of $C^{14}O_2$ in e/e generally remained greater than that in +/e, which in turn remained greater than that in +/+ until a background level was reached as late as 46 hours after injection.

Counts of pupal sheaths of flies injected with β-alanine-1-C^{14} showed e/e to have much lower C^{14} counts than +/+, while counts of +/e were intermediate (Table 1). Counts of wings and body extracts, on the other hand, showed that e/e had more activity than +/+, while counts of +/e were intermediate. The wing counts of flies injected with β-alanine-1-C^{14} were insignificant.

These results indicate that ebony homozygotes, instead of storing much C^{14} derived from β-alanine in pupal sheaths, deposit it in the adult body and wings, and decarboxylate the amino acid, and excrete C^{14}-labeled CO_2 rapidly immediately after injection. They then oxidize the remainder of the molecule (at least the second carbon) so that additional radioactive CO_2 is excreted at a later period. Non-ebony homozygotes, on the other hand, store much more C^{14} derived from β-alanine in pupal sheaths, subsequently deposit less in the body and wings, and decarboxylate and oxidize less of the amino acid. Heterozygotes are intermediate in all these activities.

Table 1. Counts of C^{14} in wings, body extracts, and pupal sheaths of homozygous ebony (e/e), homozygous non-ebony (+/+), and heterozygous (+/e) *Drosophila melanogaster* females injected, as newly formed pupae, with C^{14}-labeled β-alanine.

Genotype	Activity (count/min per fly)		
	Wings	Body extracts	Pupal sheaths
β-Alanine-1-C^{14} (66 in each genotype)			
e/e	0.6	6.2	14.4
+/e	0.8	5.4	817.4
+/+	0.7	4.0	882.8
β-Alanine-2-C^{14} (52 in each genotype)			
e/e	17.3	112.0	185.3
+/e	8.3	46.0	1071.3
+/+	4.3	29.0	1102.6

It is not known what, if anything, these activities have to do with deposition of dark pigment in the body wall. It is interesting that the heterozygote, which is intermediate in the metabolic steps studied here, is also somewhat intermediate in degree of body darkening, though heterozygotes are sometimes indistinguishable from non-ebony homozygotes when cultured at 25°C. Also, the heterozygote shows a competitive advantage over the homozygous types (*1–3*). Perhaps the heterozygote has a greater range of enzyme activity than homozygous types do; this is a theory postulated currently by students of heterosis and genetic homoestasis.

Drosophila virilis may show similar differential deposition of β-alanine. Seki (*5*) in chromatographic studies of homogenized pupal sheaths of this species, noted an absence of β-alanine among ebony homozygotes, while the wild type showed appreciable amounts of this amino acid (*6*).

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14 January 1963