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10. Bone marrow aspirate or peripheral blood was collected in heparin (100 unit/ml) in sterile plastic tubes (Falcon No. 2001) and allowed to settle for 2 hours at 37°C. The plasma cell layer is aspirated and washed in medium 199 containing 10 percent γ-globulin-free pooled human AB serum.
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12. F. Daguillard, D. C. Heiner, M. Richter, B. Rose, *Clin. Exp. Immunol.* **4**, 203 (1969). Cells were cultured in 1-ml volumes containing 1×10^6 cells suspended in medium 199 containing 10 percent serum (*10*) for 5 days in a humid atmosphere of 4 percent CO_2 in air at 37°C. One microcurie of tritiated thymidine (New England Nuclear; specific activity 6.7 c/mmole) was added 16 to 18 hours before termination of the culture. At the beginning of culturing 100 μg of the specific antiserum to immunoglobulin (*13*), 100 μg of normal goat serum, or 0.1 ml of the proper dilution of either PHA or PWM are added to separate culture tubes.
13. P. J. McConahey and F. J. Dixon, *Int. Arch. Allergy* **29**, 185 (1966). One milligram of monospecific purified goat antiserum to human Ig (Hyland) was conjugated to 1 mc of ^{125}I (International Chemical and Nuclear). The binding of the ^{125}I-labeled antiserum to Ig to the cells was achieved by incubating 1×10^6 cells with 100 μg of the labeled antiserum at 4°C for 2 hours under continuous gentle shaking. Cells were then washed three times and the radioactivity was counted in a gamma counter.
14. All the antiserums to immunoglobulins were tested for monospecificity by immunodiffusion and by their capacity to agglutinate tanned sheep red cells coated with purified immunoglobulins. To test for the specific antibody content of the antiserums to IgA or IgG, they were adsorbed with specific immunoadsorbents according to the method of Avrameas and Ternynack (*15*). The adsorbed antibody was eluted, tested for protein content, and then sterilized by Millipore filtration (0.45 μm pore size) before use.
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16. Supported by rheumatology Veterans Administration training grant TR-39. Part of the work was performed at the Allergy and Immunochemistry Division, Royal Victoria Hospital, Montreal, Canada, and was supported by the Medical Research Council of Canada. The technical assistance of C. LeClaire and J. Kelly is acknowledged.

8 June 1971; revised 26 July 1971

L-Leucine: A Neuroactive Substance in Insects

Abstract. *A compound was isolated from the blood of silkworm larvae,* Bombyx mori, *which had been prostrated with DDT; this compound increased the spontaneous discharge in the isolated abdominal nerve cord of the American cockroach,* Periplaneta americana. *The compound was identified as* L-*leucine.*

Sternburg and Kearns have reported that an unknown neuroactive substance is released into the blood of American cockroaches and crayfish during DDT poisoning (*1*, *2*). We now report that a neuroactive substance was isolated from the blood of silkworms, *Bombyx mori*, that had been prostrated with DDT; the compound was identified as L-leucine.

Four thousand fifth-instar larvae of silkworms were treated with a solution of DDT in acetone (approximately 1 mg of DDT per larva) and bled after being kept at 26°C for 12 hours. The blood was dropped from the cut ends of prolegs into a chilled mixture of ethanol and Dry Ice. The collected blood (about 450 g) was deproteinized with chilled ethanol, which was then evaporated at reduced pressure in an atmosphere of carbon dioxide. The residue was fractionated by column chromatography (i) through cellulose powder eluted with a mixture of *n*-butanol, acetic acid, and water (4 : 1 : 5) and (ii) through Sephadex LH-20 eluted with aqueous ethanol. Each fraction was examined by paper chromatography developed with a solvent mixture [*n*-butanol, acetic acid, and water (4 : 1 : 5)] (*3*).

The assay of neuroactivity was carried out at 22° to 26°C by recording the spontaneous discharge in the isolated abdominal nerve cord of the American cockroach, *Periplaneta americana*, immersed in a physiological saline solution in a small chamber (2.5 ml). A small portion of the cord between the fifth and sixth ganglia was kept out of the solution to place on a recording Ag-AgCl electrode (100 μm in diameter) in the air. An indifferent electrode was placed in the solution. The test sample dissolved in a saline solution was applied into the chamber.

A neuroactive fraction contained three substances (R-1, R-2, and R-3), which were positive to ninhydrin and diazotized *p*-nitroaniline and had R_F's of 0.62, 0.58, and 0.48, respectively. Substances R-1 (6 mg) and R-3 (6 mg) were isolated in colorless crytalline state by fractional crystallization. The purified R-3 did not show any neuroactivity, but R-1 was highly active (Fig. 1a). Substance R-1 resembled the substance reported by Sternburg and Kearns (*2*, *4*) with respect to its action on the insect nervous system and to its chromatographic behavior, except for the positive ninhydrin test; but by paper chromatography it was different from some known neuroactive substances such as acetylcholine, dopa, dopamine, adrenaline, noradrenaline, 5-hydroxytryptamine, glutamic acid, and γ-aminobutyric acid.

Substance R-1 decomposed at 250° to 251°C. It did not show any characteristic ultraviolet absorption spectrum. The mass spectrum of R-1 had a small peak at *m/e* (mass/charge) 131, strong ones at 86, 74, 44, and 43 and a moderate one at 57. They could be assigned as the molecular ion (M^+),

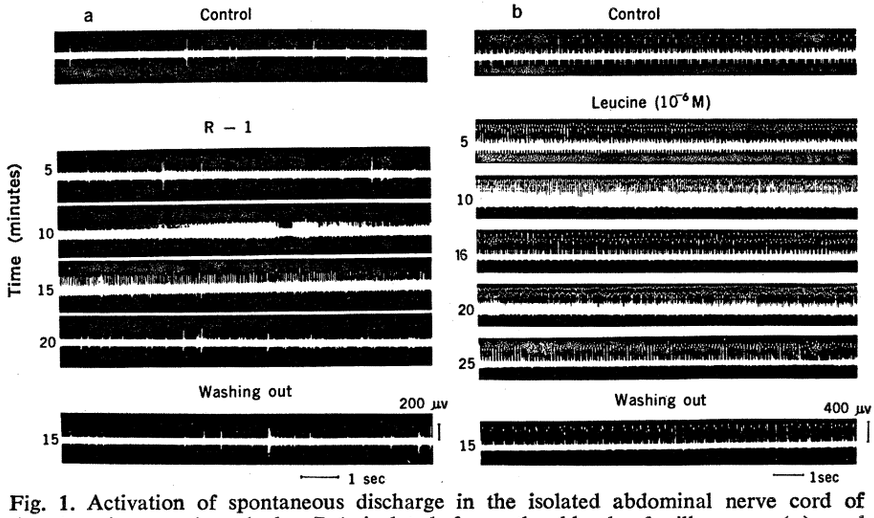

Fig. 1. Activation of spontaneous discharge in the isolated abdominal nerve cord of the American cockroach by R-1 isolated from the blood of silkworms (a) and authentic L-leucine at $10^{-6}M$ (b).

M+ −COOH, M+ $-C_4H_9$, CO_2+, C_3H_7+, and C_4H_9+, respectively. This pattern suggested that R-1 was leucine. Comparison of R-1 with an authentic sample of L-leucine showed they were identical in respect to mass and infrared spectra and paper chromatography. The commercially available pure sample of L-leucine showed similar neuroactivity at $10^{-6}M$ as shown in Fig. 1b. With both R-1 and L-leucine, the highest activity appeared about 10 minutes after treatment. Thus, the neuroactive substance isolated from the blood of silkworms prostrated with DDT was identified as L-leucine. This is the first evidence for the neuroactivity of L-leucine in insects.

Substance R-3 was identified as tyrosine by paper chromatography and spectrometry. Substance R-2 could not be isolated but was presumed to be isoleucine from its R_F value. Neither the authentic sample of L-tyrosine nor that of L-isoleucine showed the neuroactivity.

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5. The electrophysiological experiments were carried out at the Department of Physiology, Kyushu University, under the direction of Professor T. Tomita. We thank Professors K. Maekawa and H. Kuriyama for advices and discussions; and Miss T. Inatsu for the mass spectrometry measurements.

24 September 1971

Ethnic Differences in Alcohol Sensitivity

Abstract. *Japanese, Taiwanese, and Koreans, after drinking amounts of alcohol that have no detectable effect on Caucasoids, respond with a marked facial flushing and mild to moderate symptoms of intoxication. Group differences are present at birth, and are probably related to variations in autonomic reactivity.*

The lower incidence of alcoholism among certain Mongoloid as compared to that in Caucasoid (*1*) groups is generally attributed to social-environmental factors. Although biological variations in alcohol sensitivity have been implicated in principle, no satisfactory evidence supports the claim that they contribute to the etiology of alcoholism (*2*). More generally, many anthropologists assume that population differences in behavior are determined almost entirely by cultural variables, and that the genetic contribution to differences in brain function and behavior among mating groups is at best trivial (*3*). An empirical demonstration of variations in physiological responses across ethnic groups would therefore be of theoretical interest, particularly if such variations had direct implications for psychological adaptation.

Having observed that many Mongoloids respond with a rapid intense flushing of the face annd with symptoms of mild to moderate intoxication after drinking alcohol in amounts that have no apparent effect on Caucasoids (*4*), I systematically compared the alcohol flushing responses of Caucasoid and various Mongoloid groups. The subjects were randomly selected, healthy Caucasoid and Mongoloid men and women between 25 and 35 years old, residing in the United States, Japan, Taiwan, and Korea, respectively. To control for cultural differences in alcohol consumption, diet, and other postnatal environmental influences, I also compared the flushing responses of healthy full-term Caucasoid infants with those of Japanese and Taiwanese infants.

Flushing was determined by optical densitometry of the earlobe and inspection of the face (*5*). Since the densitometer response was linear in the range of values tested, differences in baseline optical density due to variations in skin pigmentation probably did not affect the results.

Subjects were tested at least 2 hours after a meal. Room temperature was always maintained at a comfortable level. All adults drank beer (5 percent alcohol by volume); and Caucasoids received consistently more alcohol per unit of body weight (0.36 to 0.45 ml/kg) than Mongoloids (0.14 to 0.30 ml/kg) (*6*). During the test subjects were asked to report any subjective symptoms that might be related to drinking; afterward they filled out a short questionnaire about their weekly consumptions of alcohol, their predisposition to intoxication, and the incidence of flushing in their families. Infants were tested by giving them small amounts of port wine in 5 percent glucose solution; no side reactions were produced. Caucasoid infants again received consistently more alcohol per body weight (0.34 to 0.45 ml/kg) than Mongoloid infants (0.16 to 0.23 ml/kg).

The results indicate that 83 percent of Mongoloid adults responded with a marked visible flush and an increase of optical density greater than 5 mm (mean increase 34.3 mm; range 14 to 78 mm), whereas only 2 of the 34 Caucasoid adults (6 percent) showed any increase of optical density greater than 5 mm, and only 1 of these flushed visibly. Population differences in flushing response were statistically significant ($P < .001$; see also Table 1). Ten nonreacting Caucasoids who subsequently

Table 1. Flushing responses, and increases of optical density and pulse pressure in the earlobe, after ingestion of alcohol. In each case, the Caucasoid population is compared to a corresponding Mongoloid group. Since only records free of artifacts were tabulated, whereas mean changes of optical density were calculated for the entire subgroup, the magnitude of flushing responses among Korean subjects appears to be less than that among other Mongoloid subgroups. This conclusion is not warranted by the results.

Group	Sample size (No.)	Visible flushing (No.)	Optical density		Pulse pressure	
			Increase > 5 mm (No.)	Mean increase for total (mm)	Measurable increase (No.)	Mean increase for total (%)
Caucasoid						
Adults	34	1	2	1.1	1 (?)	5 (?)
Infants	20	1	1	1.7	0	
Japanese						
Adults	38	32*	34*	36.8†	33*	257†
Infants	25	17*	17*	16.8†	9	
Taiwanese						
Adults	24	19*	20*	37.7†	19*	246†
Infants	10	9*	9*	14.6†	4	
Korean						
Adults	20	14*	10*‡	17.4†‡	9*‡	161†

* Ethnic group differences are significant at $P < .001$, chi-square test. † $P < .001$, *t*-test. ‡The records of six Korean subjects could not be analyzed reliably because of line-voltage disturbances.