Table 2. Effectiveness of placebos in relieving pain of pathological origin. The average percentage relieved in Table 2 differs from the average percentage relieved in Table 1: $t = 9.28$ and $p = 0.0001$.

Study	Subjects (No.)	Satisfactorily relieved by placebo (%)
Severe postoperative wound		
(*17*)	118	21
(*18*)	29	31
(*19*)	34	26
(*20*) (The av. percentage relieved by placebo was 33%.)	52	40
	36	26
	44	34
	40	32
(*21*) (The av. percentage relieved by placebo was 39%.)	14	50
	20	37
	15	53
	21	40
	15	40
	15	15
Pain from angina pectoris		
(*22*)	66	38
(*23*)	19	26
(*24*)	27	38
Pain from metastatic disease		
(*25*)	67	42
Headache		
(*26*)	199	52
Totals (10 studies):	831	Av. 34.6±2.9

ings) is not enough. Thus there is exposed in this new framework unsuspected ties between mind and body, revealed by the study of drug action.

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References

1. H. K. Beecher, *Am. J. Physiol.* **187**, 164 (1956).
2. R. A. Cleghorn, B. F. Graham, R. B. Campbell, N. K. Rublee, F. H Elliott, M. Saffran, "Anxiety States: Their Response to ACTH and to Isotonic Saline," in *Proceedings First Clinical ACTH Conference* (Blakiston, Philadelphia, Pa., 1950), pp. 561–5.
3. H. K. Beecher, *The Measurement of Subjective Responses*: Quantitative Effects of Drugs, (Oxford Univ. Press, New York, 1959).
4. F. B. Benjamin, *Science* **128**, 303 (1958); personal communication, 1959.
5. S. Flodmark and T. Wramner, *Acta Physiol. Scandinav.* **9**, 88 (1945).
6. C. T. Javert and J. D. Hardy, *Anesthesiology* **12**, 189 (1951).
7. H. G. Wolff and H. Goodell, *Research Publs. Assoc. Research Nervous Mental Disease* **23**, 434 (1943).
8. H. G. Wolff, J. D. Hardy, H. Goodell, *J. Clin. Invest.* **19**, 659 (1940).
9. J. E. Denton, O. H. Straus, H. K. Beecher, unpublished data, 1949.
10. J. D. Hardy, H. G. Wolff, H. Goodell, *J. Appl. Physiol.* **5**, 247 (1952).
11. J. G. Foulks and E. E. Daniel, *Can. J. Biochem. and Physiol.* **35**, 455 (1957).
12. E. A. Gaensler, *J. Clin. Invest.* **30**, 406 (1951).
13. H. E. Hill, C. H. Kornetsky, H. G. Flanary, A. Wikler, *ibid.* **31**, 473 (1952).
14. D. I. Macht, N. B. Herman, C. S. Levy, *J. Pharmacol. Exptl. Therap.* **8**, 1 (1916).
15. I. B. Harrison and N. H. Bigelow, *Research Publs. Assoc. Research Mental Nervous Disease* **23**, 154 (1943).
16. A. J. H. Hewer and C. A. Keele, *Lancet* **1948, 2**, 683 (1948).
17. A. S. Keats and H. K. Beecher, *J. Pharmacol. Exptl. Therap.* **100**, 1 (1950).
18. H. K. Beecher, P. A Deffer, F. E. Fink, D. B. Sullivan, *U.S. Armed Forces Med. J.* **2**, 1269 (1951).
19. A. S. Keats, G. L. D'Alessandro, H. K. Beecher, *J. Am. Med. Assoc.* **147**, 1761 (1951).
20. H. K. Beecher, A. S. Keats, F. Mosteller, L. Lasagna, *J. Pharmacol. Exptl. Therap.* **109**, 393 (1953).
21. L. Lasagna, F. Mosteller, J. M. von Felsinger, H. K. Beecher, *Am. J. Med.* **16**, 770 (1954).
22. W. Evans and C. Hoyle, *Quart. J. Med.* **2**, 311 (1933).
23. J. Travell, S. H. Rinzler, H. Bakst, Z. H. Benjamin, A. L. Bobb, *Ann. N. Y. Acad. Sci.* **52**, 345 (1949).
24. T. Greiner, H. Gold, McK. Cattell, J. Travell, H. Bakst, S. H. Rinzler, Z. H. Benjamin, L. J. Warshaw, A. L. Bobb, N. T. Kwit, W. Modell, H. H. Rothendler, C. R. Nesseloff, M. L. Kramer, *Am. J. Med.* **9**, 143 (1950).
25. R. Houde and S. L. Wallenstein, *Drug Addiction and Narcotic Bull.*, Appendix F, 660 (1953).
26. E. M. Jellinek, *Biometrics* **2**, 87 (1946).

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A Function for Ascorbic Acid in the Metabolism of an Insect

Abstract. Homogenates of *Blattella conjuncta* oxidize L-tyrosine. The reaction is diminished by dialysis, but may be reactivated with L-ascorbic acid. Glutathione, pyridoxal phosphate, and folic acid also activate the system.

It has been demonstrated (*1–3*) that ascorbic acid will activate the L-tyrosine oxidase system of mammalian liver. However, the function of the compound in insect metabolism is unknown. Previous investigations (*4*) have demonstrated that the cockroach contains large amounts of ascorbic acid. In fact, the concentration in the whole insect (10 to 19 mg/100 gm) greatly exceeds that in whole guinea pigs [2.4 mg/100 gm (*5*)]. This concentration presumably indicates that ascorbic acid plays an important role in insect metabolism. The present study was undertaken to study tyrosine oxidation by insect tissues which, by analogy with the process in mammalian tissues, would be expected to require ascorbic acid.

Blattella conjuncta was captured locally in the Upper Hutt Valley, near Wellington, New Zealand. The adult insects were killed by decapitation, and the whole insects were homogenized with two volumes of ice-cold 0.14*M* potassium chloride solution in a Potter-Elvehjem apparatus. Insoluble materials were removed by centrifugation at 2°C. The supernatant was adjusted to *p*H 7.5 and dialyzed in a cellophane bag against 0.14*M* potassium chloride solution for 24 hours at 4°C. The resulting solution was used as the source of the tyrosine oxidase system.

Table 1. Effects of ascorbic acid and other cofactors on the tyrosine oxidase activity of a dialyzed insect homogenate. The activity is given in microliters of oxygen per hour.

Components added to homogenate	Activity
None	11
α-Ketoglutarate (α-K)	14
α-K + ascorbic acid	31
α-K + glutathione	15
α-K + pyridoxal phosphate	14
α-K + folic acid	21
α-K + ascorbic acid + glutathione	42
α-K + ascorbic acid + glutathione + pyridoxal phosphate	49
α-K + ascorbic acid + folic acid	37
α-K + ascorbic acid + glutathione + pyridoxal phosphate + folic acid	50

Activity was determined by Warburg techniques at 37°C in air with sodium hydroxide papers in the center wells of the vessels. Each vessel contained 1.0 ml of the dialyzed solution, 1.0 ml of 0.2*M* phosphate buffer at *p*H 7.2, and 6 μmole of L-tyrosine. The side arm contained 0.1 ml of 0.1*M* α-ketoglutarate and 0.1 ml of a solution of cofactors. The total volume in each vessel was 3.0 ml. The following cofactors were used in some experiments: ascorbic acid (1 mg), glutathione (1 mg), pyridoxal phosphate (10 μg), and folic acid (200 μg). The reaction was initiated by tipping the side arm. Manometric readings were taken for 1 hour.

The results of a typical experiment are given in Table 1. It is clear from these figures that the tyrosine oxidase system is activated by ascorbic acid. It is also activated by folic acid. The greatest effect results from additions of ascorbic acid, glutathione, and pyridoxal phosphate.

It is clear that the oxidation of L-tyrosine by homogenates of this insect probably follows a pathway similar to that in mammals. It is known that the latter deaminate tyrosine by a transamination reaction with α-ketoglutarate which requires pyridoxal phosphate (*6*). The resulting *p*-hydroxyphenylpyruvic acid is oxidized to homogentisic acid and carbon dioxide. This process involves 2,5-dihydroxyphenylpyruvic acid as an intermediate and requires ascorbic acid and glutathione. The effect of folic acid on the system is difficult to explain, but a similar effect has been reported for the mammalian system and it has been suggested that the stimulation is not due to a direct effect of the compound on the enzymes (*2*).

The present study clearly establishes a function for ascorbic acid in insect metabolism.

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References

1. J. N. Williams, Jr., and A. Sreenivasan, *J. Biol. Chem.* **203**, 605 (1953).
2. ——, *ibid.* **203**, 613 (1953).
3. W. E. Knox and M. LeMay-Knox, *Biochem. J.* **49**, 686 (1951).
4. E. Wollmann, A. Giroud, R. Ratsimamanga, *Compt. rend. soc. biol.* **124**, 434 (1937).
5. C. M. Damron, M. M. Monier, J. H. Roe, *J. Biol. Chem.* **195**, 599 (1952).
6. Z. N. Canellakis and P. P. Cohen, *ibid.* **222**, 53, 63 (1956).

21 March 1960