override, at least temporarily, the action of the respiratory center.

The nervous mechanism controlling the action of the spleen in mobilizing reserves of hemoglobin responds therefore to only one of the three chemical stimuli which are effective for the respiratory center, viz., increase in CO2, but it is to be noted that this particular stimulus has been shown in the latter case to be by far the most important of the three. In fact, CO<sub>2</sub> is regarded as the "normal respiratory stimulant." In man, at any rate, the respiratory center responds to an addition of as little as 0.8 per cent. CO, to inspired air,7 whereas in our experiments 2.5 per cent. CO<sub>2</sub> was ineffective on the spleen. It is perhaps to be expected that the control of the spleen should be less sensitive than the control of respiration, for the action of the spleen is an emergency one and is called into play under stress, whereas the respiratory center acts as a constant regulator and consequently must be able to respond to relatively small changes.

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## THE CHEMICAL NATURE OF CYPRIDINA LUCIFERIN

THE nitrogen content of Cypridina luciferin is one of the most essential factors in deciding whether it is a protein or not. Luciferin isolated by my former method1 contains about 4 per cent. of nitrogen, whereas luciferin isolated by my recent method2 contains about 6 per cent. of nitrogen, as shown in Table I. This variability in quantity of nitrogen con-

TABLE I NITROGEN CONTENT OF CYPRIDINA LUCIFERIN\*

Isolated by tw	o methods	Former	Recent
	Exp. 1	3.58 per cent.	5.84 per cent.
One sample	" 2	3.74 '' ''	5.87 '' ''
	" 3	3.85 '' ''	6.04 '' ''
Average		3.72 '' ''	5.91 '' ''

<sup>\*</sup> For all the determinations of nitrogen I am indebted to Mr. Shigeo Okido.

tent is not clear, although the degree of purity of luciferin isolated may be supposed to be one cause. If so, the precipitation of luciferin with ammonium sulphate may act more selectively than when benzene

dissolves it. Either of these amounts of nitrogen content is, however, too small for a protein. In this connection, it should be borne in mind that luciferin isolated by both my methods gives no protein color

I have also found that Cypridina luciferin isolated by the two methods contains a large amount of phosphorus.3 On the other hand, Witte's peptone, which chiefly consists of proteoses,4 and Merck's peptone are found to contain phosphorus. In respect of phosphorus content, Harvey's belief<sup>5</sup> that Cypridina luciferin is a proteose or peptone seems to be favored. But this is not the case. Neither Witte's nor Merck's peptone is soluble in benzene, chloroform, ether, etc., as would be expected, whereas Cypridina luciferin is readily soluble in these solvents. These solubility relations of luciferin entirely refute the idea of Harvey.

The evidences from these triple experiments of nitrogen and phosphorus contents and solubility indicate that Cypridina luciferin is of a chemical nature similar in type to that of phospholipids and in particular to that of lecithins. The amount of nitrogen contained in luciferin isolated by my methods, however, is too much for a lecithin in the present state of purification.6 We should, therefore, direct our research to the discovery of a pure luciferin, as pure as a phospholipid.

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3 It was recently found that the luminous organs of the larva, pupa and adults of the Japanese firefly, Luciola cruciata (vitticollis), contain phosphorus.

<sup>4</sup> A. P. Mathews, "Text-book of Physiological Chemistry," 1927, p. 992.

5 E. N. Harvey, Journ. Gen. Physiol., 1: 269-293,
1919; Bull. Nation. Res. Coun., 59: 50-62, 1927.
6 Cypridina luciferin isolated by any methods contains

some sulphur, which may be one of the impurities.

<sup>&</sup>lt;sup>7</sup> E. H. Starling, op. cit., p. 875.

<sup>&</sup>lt;sup>1</sup> S. Kanda, Am. Journ. Physiol., 68: 435-443, 1924. <sup>2</sup> S. Kanda, Sci. Pap. Inst. Phys. Chem. Res., 10: 91-

<sup>98, 1929;</sup> also Chem. News, 138: 247-248, 258-260, 1929.