The dispersing tubes are thirty-five millimeters in diameter and twenty-two centimeters long, and are closed by No. 7 solid rubber stoppers. Their capacity is about 160 cubic centimeters, which permits eighty cubic centimeters of water to be used in dispersing. The sedimentation tube consists of a glass tube which fits into a glass cup by a ground joint. The cup is three centimeters high and the tube nineteen centimeters, and both are made from forty-millimeter tubing. The ground joint is fifteen millimeters in length, and if the tube is twisted firmly into the cup the apparatus will not leak, even after standing full of water for several days. A hole in a wood block is used for a support, but the cup can be made with a base so that it will stand of itself.

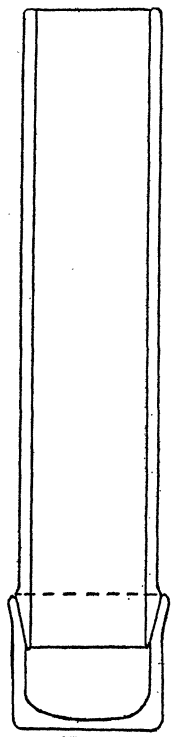

FIG. 1

The sediments are transferred from the dispersing tube to the sedimentation tube by removing one rubber stopper, inserting the dispersing tube into the inverted sedimentation tube, righting the whole apparatus, removing the other rubber stopper and washing with a stream of water. The silts and clays are decanted from the sedimentation tube, the cup in which the sands have collected is removed from the tube and the sands are readily washed out from the cup with a jet from a water bottle. The whole process is simple and very rapid. In fact, other processes involving decantation could probably be facilitated by a sedimentation tube of this type.

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STOCK CULTURES OF AMEBA

IN the course of experiments in this laboratory it has been necessary to maintain cultures of *Ameba proteus* in stock. The writer endeavored to find a medium that made requisite a minimum amount of attention. The effort in this direction met with considerable success, as appears below. In view of the wide use of *Ameba* of the *proteus* type in biological research and elementary instruction in biology, a culture medium that is simple, reproducible and extremely reliable will be of general interest. The medium used is as follows:

NaCl	0.1 gr.
KCl	0.004 gr.
$CaCl_2$	0.006 gr.
H_2O	1000 cc (glass distilled)

Two hundred to 250 cc of this solution is put into a finger-bowl or glass crystallizing dish of 8 or 10 cm diameter and to each of such dishes is added 4 or 5 grains of polished rice (any brand carried at the corner grocery is suitable). The cultures thus prepared are immediately seeded with fifty to one hundred amebas, covered with glass plates to prevent evaporation and entry of dust and then left, best in a dark cool place, to develop. Such cultures will produce a fine crop in from two to four weeks and so far in some thirty or forty cultures the writer has had only one or two failures. There are at present on the shelves of the laboratory, out of five that were set up as a test, three cultures one year old that have ample numbers of amebas; the other two died out in eleven months.

These five cultures during their existence have been deliberately neglected. No detritus was removed. Rice was added only when it was noticed that none was apparent in the culture. Water too has been added to compensate for evaporation with no attempt at regularity, say, on the average once a month. The temperature variation has been from 19 to 28° C.

In other words, the cultures have been subjected to as careless handling as if in the hands of a somewhat below par student assistant, but they have survived.

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SPECIAL ARTICLES

THE CHEMICAL CONTROL OF SPLENIC CONTRACTION

THE behavior of the spleen in responding to different physiological requirements and the fact that this response occurs only when its nerve supply is intact[1] suggests control by some center. At times when there is an emergency call for hemoglobin, as in asphyxia, hemorrhage or severe muscular exercise, contraction of the spleen throws into circulation a large number of red blood cells, as many as one third the total number in the body.[2] When the period of stress is over the spleen relaxes and the excess red blood cells are withdrawn from circulation. The

[1] E. A. Schäfer and B. Moore, *Journ. Physiol.*, 20 (1): 1–50, 1896.

[2] E. H. Starling, "Principles of Human Physiology," fifth edition, p. 820, 1930.

regulatory nature of the reaction and the character of the stimuli which set it off suggest at once a parallel with the action of the respiratory center. The stimuli acting on the respiratory center are (1) variation in the CO_2 pressure in the blood, (2) lack of O_2, (3) increase in the H-ion concentration of the blood, which in the normal animal is chiefly brought about through the presence of lactic acid. According to Gesell's now well-known theory, excitation of the cells in the respiratory center is the result of a shift in the equilibrium between these cells and their environment. It is evidently the change in the H-ion concentration within the cells which is the all-important internal factor, and because of its penetrating power CO_2 may be considered the chief environmental factor.

We have determined the effect on the spleen of these three stimuli: (1) increase in CO_2, (2) decrease of O_2 and (3) addition of lactic acid to the blood. For this purpose we have employed curarized, decapitate cats,[3] recording changes in the area of the spleen by tracing its outline on glass plates.[4] The advantage of such a preparation is that with the entire brain gone there can be no effect from emotional states, such as is seen in animals with exteriorized spleens,[5] or from muscular movements such as occur in decerebrate preparations,[4] both of which conditions would cause fairly rapid changes in the size of the spleen and confuse the issue. Furthermore, the administration of different gas mixtures is readily possible because of the necessity of supplying artificial respiration. This in itself is an added advantage because constant ventilation is assured regardless of the nature of the gas mixture, a condition which would certainly not obtain were the animal breathing on its own with medulla intact.

(1) A mixture of 4.8 per cent. CO_2 in O_2 caused a 10 per cent. reduction in the area of the spleen within five minutes (three experiments). There was no further significant change after ten minutes or even twenty minutes. Seven per cent. CO_2 in O_2 gave exactly the same results (five experiments). Eleven and eight tenths per cent. CO_2 in O_2 caused a 20 per cent. reduction in five minutes, and 30 per cent. within ten minutes, practically the maximum contraction or the post mortem condition (four experiments).

Similar results were obtained when the CO_2 was mixed with air instead of O_2. Two and one half per cent. CO_2 in air produced no significant change (two experiments). Five and six tenths per cent. CO_2 in air caused in five minutes a 10 per cent. reduction in the area of the spleen, with no further change after ten minutes or twenty minutes (three experiments). Ten and five tenths per cent. CO_2 in air caused a 14 per cent. reduction in five minutes (two experiments). Although fourteen different cats were used in the nineteen experiments, there was excellent agreement of the data and no experiment gave results contradictory to those of the others.

(2) Administration of 9 per cent. O_2 (three experiments) or even 5.1 per cent. O_2 (four experiments) in N_2 had no effect on the size of the spleen even after twenty minutes. The post mortem contraction following death by asphyxiation was normal in each case, therefore lack of response to lowered oxygen tension could not have been due to inability on the part of these spleens to respond to an adequate stimulus.

(3) Human blood may contain after severe muscular exercise as much as 0.12 per cent. lactic acid, or in exceptional cases 0.20 per cent.[6] If we consider the blood to constitute 7 per cent. of the body weight, then injection of 140 mgm lactic acid per kilo body weight would correspond to the exceptional cases, and injection of half this amount would correspond to the effect of severe exercise. The latter dose therefore should be sufficient to bring about contraction of the spleen if the lactic acid produced during muscular exercise is an adequate stimulus for such contraction. In order to affect the heart as little as possible and to prevent too great hemolysis of the blood by the sudden addition of a comparatively large volume of acid, we injected the requisite amount of 5 per cent. lactic acid sometimes directly into the femoral vein, and at others into a small side branch opening into the femoral vein, very slowly, *i.e.*, over a period of about two minutes. We found that when the larger dose was given the animal died immediately and the spleen contracted at once to its post mortem size (two experiments). On the other hand, when the lighter dose was given there was no change in the size of the spleen, and the preparation remained alive and in good condition (three experiments). In one case half an hour after the injection of 70 mgm of lactic acid per kilo, the size of the spleen having remained unchanged, 10.6 per cent. CO_2 in air was administered. The area of the spleen now diminished by 15 per cent. in 5 minutes, exactly as in the untreated preparation. In the other two cases the post mortem contraction was normal.

It should be emphasized that in this paper we are dealing only with the chemical stimuli affecting the adjustments of the spleen. Stimuli from the emotional (higher) nerve centers together with the action of epinephrine cause profound changes in the size of the spleen.[5] Similarly emotion and volition may

[3] S. F. Cook and J. M. D. Olmsted, *Am. Journ. Psysiol.*, 92 (1): 249–252, 1930.

[4] S. F. Cook and M. I. Rose, *ibid,* 92 (1): 240–248. 1930.

[5] J. Barcroft, *Journ. Physiol.*, 58 (4): 375–382, 1930.

[6] A. V. Hill, "Muscular Activity," p. 87, 1926.

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 CO_2, 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_2 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_2 to inspired air,[7] whereas in our experiments 2.5 per cent. CO_2 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 method[1] contains about 4 per cent. of nitrogen, whereas luciferin isolated by my recent method[2] contains about 6 per cent. of nitrogen, as shown in Table I. This variability in quantity of nitrogen content 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 tests.

TABLE I

NITROGEN CONTENT OF CYPRIDINA LUCIFERIN*

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

* For all the determinations of nitrogen I am indebted to Mr. Shigeo Okido.

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[5] 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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[7] E. H. Starling, *op. cit.*, p. 875.

[1] S. Kanda, *Am. Journ. Physiol.*, 68: 435–443, 1924.

[2] S. Kanda, *Sci. Pap. Inst. Phys. Chem. Res.*, 10: 91–98, 1929; also *Chem. News*, 138: 247–248, 258–260, 1929.

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

[4] 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.

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