All three of the fluidextracts show a distinct deterioration regardless of the hydrogen-ion concentration. The various hydrogen-ion concentrations apparently had no influence in preventing the deterioration.

TABLE V.—ORIGINAL ASSAY = 150% SOLUTION OF ERGOT FOR HYPODERMIC USE.

Sample No.	Date Made.	p_H.	Date Assayed.	Broom and Clark (Per Cent.)
1	3-11-29	5.50	3-10-30	1%
2	3-11-29	4.50	3-11-30	1.25%
3	3-11-29	3.90	3-12-30	1.66⅔%
4	3-11-29	3.30	3-14-30	2.5%
5	3-11-29	3.00	3-18-30	40.0%
6	3-11-29	2.50	3-21-30	10.0%
7	3-11-29	2.20	3-24-30	5.0%
8	3-11-29	1.80	3-30-30	2.0%

Considering the results as shown in Tables IV, V, VI and Charts I and II: (1) Not all Fluidextracts of Ergot are influenced by the hydrogen-ion concentration in preventing deterioration. (2) For some Fluidextracts of Ergot the hydrogen-ion concentration of around 3.00 appears

TABLE VI.—ORIGINAL ASSAY = 200% SOLUTION ERGOT FOR HYPODERMIC USE.

Sample No.	Date Made.	p_H.	Date Assayed.	Broom and Clark (Per Cent.)
1	1-16-29	4.30	9-15-29	5%
2	1-16-29	3.20	9-16-29	17%
3	1-16-29	2.90	9-20-29	80%
4	1-16-29	2.40	10- 7-29	80%
5	1-16-29	1.90	10- 9-29	33⅓%
6	1-16-29	1.75	10-11-29	25%

to be the critical point where there is the least deterioration. (3) The passing of a current of air through a fluidextract appears to be influenced by the hydrogen-ion concentration in preventing deterioration. (4) The hydrogen-ion concentration appears to have no influence on a fluidextract if frequently exposed to air or subjected to excess heat or temperature.

(*To be continued*)

TRANSPARENT LIFE STUDIES.*

2. EFFECT OF STRYCHNINE UPON DAPHNIA.[1]

BY ARNO VIEHOEVER AND ANNA SCHWENK MIKURIYA.

The remarkable suitability of certain transparent organisms as Daphnia, an ideal representative of transparent life (1), for purpose of demonstration of major life functions, namely, the action of the muscular, glandular and nervous system, has been pointed out before by one of us (2). Equally the use of this or other transparent animals for testing drugs was suggested. To demonstrate that the effect of stimulants and depressants and other therapeutic agencies can be quantitatively checked, many experiments were carried out, others are under way. The results obtained with a soluble salt of the alkaloid strychnine on Daphnia magna will be first recorded here.

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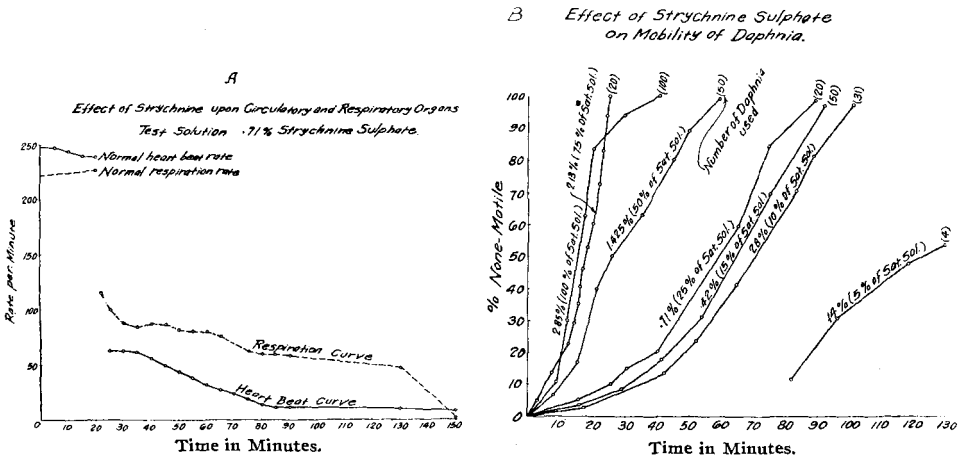
* From the Biochemical Research Laboratories of the Philadelphia College of Pharmacy and Science. The studies have had the initial support of the AMERICAN PHARMACEUTICAL ASSOCIATION and are continued with the generous help of Dr. Kilmer, New Brunswick, supporting the Kilmer Fellowship.

[1] Scientific Section, A. PH. A., Miami meeting, 1931.

General Physiological Effects.—The general effect of strychnine upon Daphnia is that of the well-known tonic or stimulating action of therapeutic small doses, the convulsive action of larger toxic, and the paralyzing action of fatal doses.

In a weak strychnine solution (containing 0.01% of strychnine sulphate) peristalsis is very marked and very rapid. The first noticeable effect upon the Daphnia placed in solutions weaker than 1% strychnine sulphate is the swimming of the animals in circles, due to paralysis of the left or the right large front swimming leg, used so prominently for locomotion. Normally the movements of the swimming antennæ or legs vary somewhat: For example, a rate of 88, 79, 71, 69 was observed, but the normal rate will be maintained within certain individual limits. However, when the Daphnias were placed in a 1% solution of strychnine sulphate, the rate of movement of the swimming legs increased to 228 per minute at the end of 15 minutes' contact in the poison, thus showing greatly increased, if not convulsive motion. Equally, intermittent body vibration was observed in this 1% solution. The following detail effects were noted in our records and may serve here for further illustration:

Animal: Daphnia. Environment: Culture water, containing 1% strychnine sulphate. Temperature 22° C.



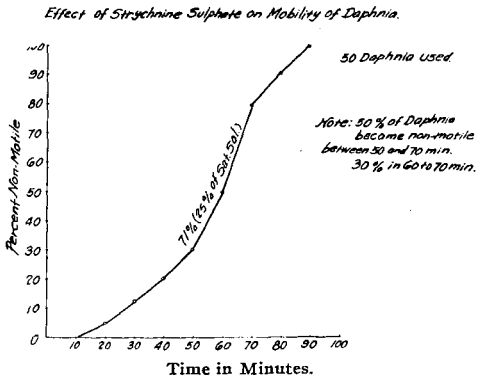
TIME OF CONTACT.

After 1 minute	The Daphnia swims on back, its whole body begins to vibrate rapidly.
After 10 minutes	Swimming on back, continued.
After 15 minutes	Summersaulting motion.
After 25 minutes	Swimming head down with occasional rise to surface.
After 30 minutes	Swimming entirely head down.
After 45 minutes	Daphnia weak, lies on one side, flaps antennæ legs on one side, cannot raise legs above head.
After 60 minutes	Rapid vibration of body discontinued, animal swims on back, movements are slow, laborious.
After 80 minutes	Heart beat is greatly reduced; 10 strong beats per minute, liver is shrunken at the ends.
	Swimmeret breathing organs move much slower than normally, now only 98 times per minute.
After 90 minutes	Daphnia immobile; the swimmeret breathing legs move at rate of 40 per minute, Heart beats 5 times per minute; eye lenses are enlarged.

Effect of Strychnine upon Breathing Organs and the Heart.—The movement of foliaceous breathing organs or transformed swimmeret legs, absorbing the oxygen from the liquid environment, can be readily observed and the speed recorded. The rate, though intermittent, is usually less than the heart beat per minute. In a solution containing 0.712% of strychnine sulphate the heart beat is more effected than the rate of respiration. The results are recorded by Graph A.

Effect of Strychnine upon Body Mobility.—To observe the relative effect of strychnine solutions containing various amounts of strychnine sulphate, such liquids were prepared. A saturated (100%, so called) solution with 2.85%; a 75% saturated solution with 2.13%; a 50% saturated solution with 1.42%; a 25% saturated solution with 0.712%; a 15% saturated solution with 0.42%; a 10% saturated solution with 0.28%; a 5% saturated solution with 0.14% of strychnine sulphate. Usually Daphnias in large number were placed in these solutions of known strength and the experiments observed in special flat test-tubes, ending into a narrow bottom. Rather than using the time of death as a degree of toxicity, we determined the time when the Daphnias were no longer capable of body mo-

C



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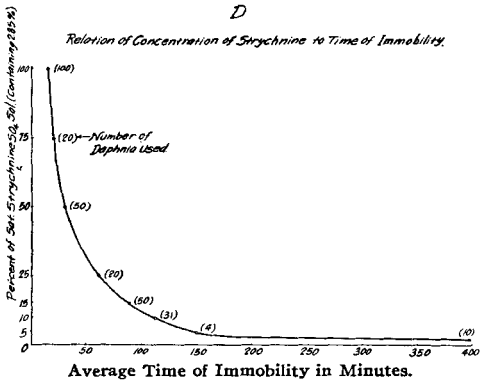

bility; that is, no longer able to swim up in the tube for oxygen or floating food supply, though certain body organs were still functioning, as the breathing and circulatory organs and, very weakly, the swimming legs. The animals usually were taken from the same strain, originally obtained from one female animal.

Graph B shows the results of these tests; the time when the Daphnias became non-motile was blotted against the number in % of animals that became non-motile.

Graph C shows a check experiment carried out with a 0.712% solution of strychnine sulphate, a day later than the experiments carried out with a solution of the same strength, 20 animals having been used in the first, 50 Daphnias in the second experiment. The graphs check remarkably well.

Graph D, shows, as did Graphs B and C, that immobility, a sign of toxic inability, is definitely dependent upon concentration of poison and time of action. The relation is expressed, however, not in a straight line, as, relatively, immobility is much quicker produced in more concentrated solutions of strychnine.

Of significance were the experiments carried out with 150 Daphnias from 5 different tanks. In a 1% strychnine solution the first case of immobility was ob-

served in 16 minutes; in the last case in 120 minutes; in 60 minutes, however, the majority of Daphnia lost their ability of body locomotion. An interesting biometric curve was obtained from these data.

CONCLUSIONS.

1. Transparent animals, and especially *Daphnia magna* are well suited for the qualitative and quantitative examination of general physiological effects upon the animal organism and its individual organs.

2. The general effect of strychnine upon Daphnia is that of the well-known tonic or stimulating action of small, therapeutic doses; the convulsive action of larger, toxic doses; the paralyzing action of fatal doses, one effect overlapping the other, depending upon particular organs or the difference in vitality of different organisms. Most striking were the effects upon organs of the muscular system; the liver, in agreement with observations recorded for more complex animals, also was visibly effected, through shrinking (3).

3. In a 0.712% solution of strychnine sulphate the heart beat was more effected than the rate of respiration.

4. The time when body immobility (the inability to rise from the bottom of flat test-tubes) is observed in the majority of animals may be used more conveniently—and much more speedily—than the time of death— as a judgment of degree of toxicity.

5. Immobility as well as death are definitely dependent upon concentration of poison in solution and time of action; results are relatively much quicker observed in concentrated than in dilute solutions of strychnine sulphate.

REFERENCES.

(1) "Transparent Life," by Arno Viehoever, *American Journal of Pharmacy*, 103 (1931), 252–278.

(2) "The Heart," by Arno Viehoever, *Ibid.*, 100 (1928), 718–745.

(3) "The Strychnine Group," by E. Poulsson; "Handbuch der experimentallen Pharmakologie," pages 322, 331, 392 (1920).

THE OIL FROM THE FRUIT OF MELIA AZEDARACH LINNÉ.*

BY LOYD E. HARRIS AND RALPH M. WILSON.

The oil[1] from the fruit is bitter. It is known as neem oil, Veepa oil, and Veppam fat. It has been used as an anthelmintic[2] and may be useful as a local application for treatment of rheumatism.

The constants[3] of the oil have been reported as follows: Specific gravity, 0.914, (15°); saponification value, 196.9; iodine value, 69.6; Reichert-Meissel value, 1.1; a butyro-refractometer reading of 52°.

EXPERIMENTAL.

A preliminary extraction with a series of selective solvents was made to determine something of the general composition of the fruit. The results are tabulated below: 80 Gm. of the ground air-dried fruit were used for each extraction.

* Scientific Section, A. PH. A., Miami meeting, 1931.

[1] U. S. Disp., 21st Edition, page 1215.

[2] Amer. Disp. (King), 8th Edition (1872), page 521.

[3] U. S. Disp., 21st Edition, page 1215.