valescent specimen. Also, if the first specimen is strongly positive (in this very limited series about 50% of the cases) the test would appear to offer no help in diagnosis. Case 14, in which the diagnosis for this reason could not be confirmed by the neutralization test, yielded virus by inoculation of a stool specimen.

As to the application of this test to population surveys, it seems probable that it will serve in the same way as has the monkey test, and possesses no advantages other than those resulting from economy.

13529

Physiological Observations Upon Larval Eustrongylides. III. Culture Attempts *in vitro* Under Sterile Conditions.

THEODOR VON BRAND AND WILLIAM F. SIMPSON.

From the Department of Biology, The Catholic University of America, Washington, D.C.

Many phases of the metabolism of internal parasites can be studied only with animals kept *in vitro*. Most helminths, however, live only a short time outside their host. A serious objection[1, 2] against such investigations is that the data may be derived from dying animals and may not be representative for healthy specimens. Preliminary experiments mentioned in the first paper of this series[3] showed that a larval Eustrongylides could be kept alive for long periods in sterile surroundings. The goal of the present investigation was to find a medium that would favor the survival *in vitro* of this worm and be simple enough to promise success for future chemical investigations.

Material and Methods. The worms used were larval Eustrongylides (probably *Eustrongylides ignotus*) from *Fundulus heteroclitus*. The sterile extraction of the nematodes required 2 persons. The fish was killed by pithing. It was pinned down on its back and its entire ventral surface was painted with a strong alcoholic iodine solution. The abdominal cavity was opened with sterile instruments and the worm cysts located. Their surface was sterilized by application of iodine solution, and the cyst was then torn open with two

[1] Lapage, G., *Nematodes Parasitic in Animals*, Chemical Publishing Co. of N. Y., Inc., 1938, 30.

[2] Stunkard, H. W., *J. Parasitol.*, 1940, **26**, 1.

[3] von Brand, Th., *J. Parasitol.*, 1938, **24**, 445.

sterile forceps. The worm was seized with one of the forceps and dropped immediately into the test-tube containing the sterile medium which was handled with the usual aseptic precautions by the second person. Varying in the different series, from 80 to 100% of the isolated worms remained sterile, as evidenced by the fact that the medium remained entirely clear despite incubation periods of several weeks at 37°C. In over 100 cases test-tubes with sterile sugar-broth were inoculated with medium from tubes in which worms had been kept for about 4 weeks. The broth remained clear in all cases in which the worms previously had been classified as sterile.

All media, except heat labile substances were sterilized by autoclaving at 15 lb pressure for 20 minutes. Blood serum and Tyrode's solution were passed through Berkefeld "N" filters.

The worms were kept singly in ¾-inch by 6-inch test-tubes containing 12 cc medium. They were closed with a cotton plug and parafilm, thus effectively preventing evaporation, and kept at 37.5 ± 1°C. At intervals of 4 to 8 weeks the old solution was replaced by a similar amount of freshly prepared medium. The pH of the media were measured by means of a potentiometer both at the beginning and the end of the period.

The viability of the nematodes was controlled 2 or 3 times a week during the first weeks after isolation, later on, once or twice a week. The worms remained usually curled up at the bottom of the test-tube, but showed throughout their life distinct movements, especially if subjected to strong light. A few days before death they usually extended in a few large loops through the whole medium. A nematode was considered dead when it had lost its bright red color or when it did not react with movements to light.

Results and Discussion. A summary of our results is presented in Table I. It is apparent that as basic medium Bacto broth, Bacto yeast extract, Proteose peptone and peptone were about equally well suited. The addition of between 0.5 and 1% NaCl was highly beneficial, and proved to be more effective than the use of Tyrode solution. The concentration of the organic material, too, could be varied widely; in the case of broth the optimal limits were about 0.8 to 1.6%. Dextrose in a concentration of 0.5% more than doubled the average length of life both in broth and yeast extract.

The addition of liver extract or serum, the use of agar-slants of various types covered with different fluids did not improve the survival; on the contrary, it was in general shorter.

The pH of the solutions dropped invariably during the incubation period. It can be assumed that the actual production of acids was

TABLE I.
Survival of a Larval Eustrongylides in Various Sterile Media.

Medium	No. of sterile worms	Length of life in days			pH	
		Avg	Min.	Max.	Initial	Change after 4-8 wks
Bacto-Yeast extr. .5%	5	5	4	6	6.78	?
" " " " + NaCl .5%	5	72	22	97	6.67	— .21
" " " " " " " " + glucose .5%	6	152	67	346	7.00	— .61
Proteose-Peptone 1% + NaCl .5% + glucose .5%	9	129	98	245	7.23	— .47
" " " in Tyrode + " "	7	82	38	240	7.80	— .31
Rabbit serum 1% in Tyrode + glucose .5%	17	43	13	94	8.50	— .54
Ascites fluid + glucose .5%	6	38	4	65	7.65	— .63
Endamoeba liver agar slants covered with broth .8% + NaCl .85% + glucose .5%	17	20	3	49	7.10*	?
Endamoeba liver agar slants covered with Tyrode + glucose .5% + serum 1%	11	23	3	61	8.50*	?
Agar slants (agar 2% + glucose .5% + NaCl .5% covered with yeast extr. .5% + NaCl .5% + glucose .5%)	18	123	70	248	7.13*	—1.51
Peptone 1% + NaCl .85% + glucose .5%	18	105	5	298	7.36	— .99
Bacto-broth .8% + NaCl .5%	5	64	10	105	6.70	— .10
" " " " " + glucose .5%	8	157	103	228	6.93	— .24
" " " " 1% " " "	10	114	56	200	7.17	— .65
" " " " 3% " " "	12	11	2	16	7.04	?
" .2% " " .85% " " "	13	116	45	195	6.91	—1.35
" .4% " " " " " "	11	92	21	226	7.03	—1.04
" 1.6% " " " " " "	19	148	83	284	7.17	— .56
" 2.5% " " " " " "	17	68	33	93	6.82	— .40
" .8% " " .5% " " " + Lederle's liver extr. 1%	9	77	17	121	7.07	— .63
Bacto-broth .8% + NaCl 3% + glucose .5% + Lederle's liver extr. 2%	12	12	2	14	6.88	?
Bacto-broth .8% + NaCl .5% + glucose .5% + horse serum 12%	9	15	6	72	6.39	?
Bacto-broth .8% in Tyrode + glucose .5%	14	90	30	163	7.85	— .62

*Figures represent the pH of the supernatant.

larger than indicated by the pH changes, since the materials present in the media have a considerable buffer capacity. This is indicated by a comparison of the average pH drop of 0.40 in the medium containing 2.5% broth with that of 1.35 in the medium containing 0.2% broth.

The average and maximal survival periods in the best media were considerably longer than those hitherto reported for Nematode larvae kept at body temperature (older literature in Hoeppli, Feng and Chu,[4] new data for *Haemonchus larvae* Stoll.[5] *Trichinella larvae* Lewin[6]). No definite sign of growth or development was seen, in this respect we were less successful than Stoll, but the worms did certainly not

[4] Hoeppli, R., Feng, L. C., and Chu, H. J., *Chinese Med. J.*, 1938, Suppl., **2**, 385.
[5] Stoll, N., *Growth*, 1940, **4**, 383.
[6] Lewin, A. J., *J. Parasitol.*, 1940, **26**, Suppl., 31.

become smaller, a phenomenon so apparent even at much lower temperature in other worms (Planaria) kept starving. The worms must either have been able to utilize the foodstuffs contained in the media, or the rate of their metabolism must have been exceedingly lowered. Previously published data[3] indicate that the carbohydrate reserves of the worms would last only for 23 days if the rate of metabolism remained unchanged. The observation that sugar in the medium increased the length of life *in vitro* might point towards its actual utilization. At the present time we are inclined to assume that our results approach those achieved with several pathogenic trypanosomes which in suitable media thrive in the stages characteristic for the intermediate host but do not change into the blood forms.

Summary. 1. The maximal survival *in vitro* of an individual Eustrongylides larva at 37.5°C was 346 days. The medium was Bacto Yeast extract 0.5% + NaCl 0.5% + Glucose 0.5%. 2. The maximal average survival of a series of worms was 157 days in a medium containing Bacto-broth 0.8% + NaCl 0.5% + Glucose 0.5%. 3. The molecular concentration of the media could be varied in rather wide limits without material change in the length of survival. 4. The presence of 0.5% glucose was decidedly beneficial. 5. The worms produced acids. 6. No moulting or other sign of development was observed.

13530 P

Ascorbic Acid Requirement of Individuals in a Large Institution.

M. K. HORWITT.

From the Biochemical Research Laboratory, Elgin State Hospital, Elgin, Illinois.

A three-year accumulation of data obtained from the analysis of more than 1,000 blood samples indicated that the average plasma ascorbic acid level of the patients in this hospital was less than .40 mg %. The lowest results, averaging .20 mg %, were obtained during the months of April, May and June and the highest during October and November when an average of .59 mg % was achieved. These data on ascorbic acid and others on various nutritional essentials which will be described in subsequent reports indicated that a large mental institution is an exceptional laboratory for conducting