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Preparations of Lysates from Cultures of *T. cruzi* and Their Effects on Normal and Tumor-Bearing Mice.

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(Introduced by A. F. Coca.)
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Klyueva and Roskin[1,2] have reported the production of an extract of *T. cruzi* which had a specific cancerolytic effect on experimental tumors in mice. Attempts to confirm these findings were unsuccessful.[3,4,5] The present report is a summary of experiments done in an attempt to verify the findings of the Soviet investigators employing a whole culture lysate.

Materials and Methods. *T. cruzi* cultivation and preparation of *T. cruzi* extracts: Cultures* were grown on NNN agar with an overlay of Tyrode's solution and incubated at 24°-25°C. At the height of growth, the concentration of organisms was determined as follows: A 4 mm loopful of culture was examined at 250× magnification. Fifty fields were examined and the average number of organisms per field divided by two represented the concentration of organisms in millions per ml. (Repeated comparisons with hemocytometer counts confirmed the reliability of this method.) Dilutions were based on units, one unit representing one million organisms.

The cultures were diluted with distilled water and metaphen (Abbot) so that each ml contained 4 units of *T. cruzi* lysate and metaphen in a concentration of 1:10,000. After storage at 8°C for 24 hours, the trypanosomes were found to have undergone lysis. This was confirmed by microscopic study of the diluted culture which was also examined for sterility. The culture lysates were stored at −20°C until used, except for one experiment in which they were kept at 8°C. None were kept more than 6 days. If not used in this period, the lysate was discarded.

Treatment. These lysates were injected into mice of varying strains and bearing several types of tumors. The injections were always given subcutaneously at a site as far from the tumor as possible. The mice were housed in individual jars and fed a prepared complete pellet diet.

The control animals received injections of sterile medium diluted with the same amount of distilled water and metaphen as the lysates. The number of injections and amounts of solution injected were the same for the control and treated groups.

The following observations were recorded:

(a) Body weight twice weekly.

(b) Tumor size 3 times weekly. (Two diameters were measured by calipers and a drawing representing the area of the tumor was made on the individual records maintained for each mouse.)

(c) Any change in the tumor or in the general condition of the animal was noted.

(d) At necropsy when the animal died or was sacrificed, gross and microscopic examinations of the tumor, liver, kidney, intestine, and heart were made. (Zenker's fixation—H. and E. stain.)

(e) Stool culture studies were made on the Bagg-albino strain of mice used to control subclinical enteric infections. No infections were found throughout the entire series.

Findings. To determine the presence of any toxic factors, injections of the *T. cruzi*

1 Shimkin, Michael B., Surgeon, U. S. Public Health Service, personal communication, 1947.

2 Klyueva, N. G., *Am. Rev. Soviet Med.*, IV, June, 1947, p. 408.

3 Hauschka, T., Saxe, L. H., Jr., and Blair, M., *J. Nat. Cancer Inst.*, 1947, **7**, No. 4.

4 Hauschka, T., and Goodwin, M. Blair, *Science*, 1948, **107**, 600.

5 Cohen, A. L., Borsook, H., and Dubnoff, J. W., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 40.

* Obtained from Dr. M. H. Soule, University of Michigan, and designated by us and by the National Inst. of Health, Tropical Disease Division, as the "Soule" or "S" strain.

TABLE I.
Treatment of Spontaneous Mammary Cancer Mice with *T. cruzi* Lysate.[1] Animal Strain the Bagg Albino.[2]

Exp.	Tumor size at start test	Dosage: No. of inject. (subcut.)	Total tumors		% No effect		% inhibition		% Regression		% Deaths	
			Test	Control	Test	Control	Test	Control	Test	Control	Test	Control
A	1 cm or less	45 (total 45 u.)	107	86	69	88	19	8	12	4	50	30
B	>1 cm	45 (total 45 u.)	51	51	86	88	12	10	2	2	71	52
C	1 cm or less	53 (total 15 u.)	37	33	24	54	52	27	24	19	S[4]	

[1] Controls treated with equivalent volume and dilution of sterile medium.
[2] Obtained from Mrs. F. O'Grady, Bronx, N. Y. C.
[3] Sacrificed 48 hours after 5th injection.
[4] Sacrificed.

lysate were given to normal mice and a variety of tumor-bearing mice. Only occasional evidence of liver and kidney damage was noted despite the fact that the average survival time of cancerous mice treated with *T. cruzi* lysate was less than the controls injected with sterile medium.

No gross or microscopic differences in the treated tumor-bearing animals of the A strain mice (spontaneous mammary carcinoma† and transplanted carcinoma No. 119‡) were observed as compared with the same types of tumor-bearing mice treated with sterile medium.

This was also true of the spontaneous mammary carcinoma larger than one cm in size of the Bagg-albino strain. (Table I, Experiment B.) However, in this latter strain where the same tumor was less than one cm in size there was a greater degree of inhibition in the treated groups as compared with the control groups. The per cent of these mice showing no effect was: controls 71%, treated 46%. (Combined findings of Experiments A and C, Table I.) This finding was not considered sufficiently significant to state that the lysate of *T. cruzi* exerted a specific cancerolytic effect. However, the association of this finding with the fact that the survival time of the treated tumor-bearing animals was distinctly less than that of the control tumor-bearing animals does indicate that the lysate may contain some active principle that exerts an effect on tumor-bearing animals, and that this effect was observed only in mice with tumors less than one cm in size. Whether or not this effect is a direct or indirect one is beyond the scope of this study. The differences observed could not be attributed to any nutritional effect since 80% of the animals surviving the experiment showed no weight loss. Those which lost weight were equally divided between treated and control groups, and tumor sizes of all of these latter mice were approximately the same.

In another group of Bagg-albino mice with spontaneous mammary carcinoma one cm or less, 5 daily injections were given, the dose being increased by one unit each day. Forty-eight hours after the last injection, each animal having received a total dose of 15 units, the mice were sacrificed. The results were considered comparable to those obtained in similar mice receiving 45 daily injections (See Table I).

A large series of experiments were performed using mice of the Bagg-albino strain with implanted sarcoma No. 180. This tumor

† Obtained from Dr. M. J. Shear, National Cancer Institute, Bethesda, Md.

‡ Obtained from Dr. T. S. Hauschka, Lankenau Hospital, Philadelphia, Pa.

proved to be completely unreliable, both controls and treated animals showing a high percentage of complete regressions. Of a group of 27 mice which received no injections, 19 showed complete disappearance of the implanted tumor after the tumor had reached a size of one cm in largest diameter.

These results do not confirm those of Klyueva and Roskin as given in the fragmentary reports available to us. At no time did we have detailed information regarding their methods of *T. cruzi* cultivation and lysate preparation. It is possible that the discrepancy may be due to differences in the ultimate nature of the lysate.

Summary. 1. No inhibition of tumors was noted in Bagg-albino strain mice with spontaneous mammary carcinoma over one cm in size when treated with whole culture lysate of *T. cruzi;* on the other hand in the same mouse strain with tumors under one cm in size a degree of inhibition was noted which warrants closer study.

2. The inhibition noted could not be attributed to malnutrition effects or concurrent bacterial infections.

3. No inhibition was noted in tumor-bearing "A" strain mice (spontaneous mammary carcinoma; transplanted carcinoma No. 119) when treated with *T. cruzi* whole culture lysates.

4. Survival time of treated tumor-bearing mice was less than that of control treated animals.

5. A simple method is given for a reproducible preparation of *T. cruzi* lysates.

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Propagation of Rabbit Fibroma Virus in the Embryonated Egg.*

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In view of the theoretical interest attached to the relationship of viruses to neoplasms,[1] it seems worth while to report the adaptation to growth in the embryonated egg of a virus which, if it does not cause a true tumor, is at least the etiologic agent of a closely related tumor-like condition. Adaptation of such a virus to a different and relatively simple experimental host such as the developing chick embryo may provide a means of studying the virus-tumor relationship. In fact the earliest use of the chick embryo in the study of viruses was for just this purpose, when Murphy and Rous obtained growth of a fowl sarcoma on inoculating into the chick embryo either minced tumor tissue or a Berkefeld filtrate of such tissue.[2] The virus studied here is that of rabbit fibroma isolated by Shope[3] from a growth on the foot of a wild rabbit. Passed since then in series through domestic rabbits, one strain (the so-called OA strain) has maintained its ability to produce tumors on subcutaneous or intratesticular inoculation. From this original strain there arose a variant (the so-called IA strain described by Andrews[4]) which had lost the ability to produce tumors and caused only

* Part of work reported here was carried out while the author was a National Research Council Fellow in the Medical Sciences.

1 Rous, P., *Bull. New York Acad. Med.*, 1947, **23**, 65.

2 Murphy, J. B., and Rous, P., *J. Exp. Med.*, 1912, **15**, 119.

3 Shope, R. E., *J. Exp. Med.*, 1932, **56**, 793.

4 Andrewes, C. H., *J. Exp. Med.*, 1936, **63**, 157.