was an average gain of 1.06 lbs daily for 26 days. The improvement in appearance was similar to that previously described.

The above data show that tryptophan is an indispensable amino acid for growing pigs. In addition, a purified ration has been formulated which is capable of producing a rate of growth equal to that obtained on a well-balanced mixture of natural feedstuffs.

The complete details of this experiment will be published in a later paper.

References

1. ALMQUIST, H. L. *J. Nutrition,* 1947, **34,** 543.
2. BAUER, C. D., and BERG, C. P. *J. Nutrition,* 1943, **26,** 51.
3. HEGSTED, D. M. *J. biol. Chem.,* 1944, **156,** 247.
4. HEINEMANN, W. W., ENSMINGER, M. E., CUNHA, T. J., and MCCULLOCH, E. C. *J. Nutrition,* 1946, **31,** 107.
5. HUGHES, E. H. *J. Nutrition,* 1940, **20,** 233.
6. HUGHES, E. H. *J. Nutrition,* 1940, **20,** 239.
7. HUGHES, E. H., and ITTNER, N. R. *J. anim. Sci.,* 1942, **1,** 116.
8. LUECKE, R. W., MCMILLEN, W. N., THORP, F., JR., and TULL, CAROLYN. *J. Nutrition,* 1947, **33,** 251.
9. MCROBERTS, V. F., and HOGAN, A. G. *J. Nutrition,* 1944, **28,** 165.
10. ROSE, W. C. *Physiol. Rev.,* 1938, **18,** 109.
11. ROSE, W. C. *Proc. Amer. phil. Soc.,* 1947, **91,** 112.
12. ROSE, W. C., HAINES, W. J., and JOHNSON, J. E. *J. biol. Chem.,* 1942, **146,** 683.
13. ROSE, W. C., HAINES, W. J., JOHNSON, J. E., and WARNER, D. T. *J. biol. Chem.,* 1943, **148,** 457.
14. ROSE, W. C., and RICE, E. E. *Science,* 1939, **90,** 186.
15. WINTROBE, M. M. *Amer. J. Physiol.,* 1939, **126,** 375.
16. WINTROBE, M. M., MILLER, M. H., FOLLIS, R. H., STEIN, H. J., MUSHATT, C., and HUMPHREYS, S. *J. Nutrition,* 1942, **24,** 345.

Trypanosoma cruzi Endotoxin (KR) in the Treatment of Malignant Mouse Tumors[1]

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Verification of the results of Roskin and Klyueva (*7* and several earlier papers) in producing the cancerolytic toxin, KR, from the lysed cells of *T. cruzi* was recently claimed by Malisoff (*8*). Since this mode of "biotherapy" is still looked upon by some as one of the promising leads in the treatment of cancer, our own largely negative findings are outlined here for the sake of critical comparison.

Since March 1945, 8 different strains of *T. cruzi* have been tested by us against 5 varieties of malignant tumors in over 1,300 experimental mice. The trypanosomes covered a wide range of virulence and were originally derived from various mammalian and insect hosts (man, monkey, armadillo, triatome). Included among the 8 *T. cruzi* strains were the "Soule" strain ("S" strain), on which Malisoff based his positive data, and the "Wellcome" strain ("W–BH" strain, inadvertently referred to as the "R" strain in an earlier publication, *4*). The latter material was the source of Roskin and Klyueva's allegedly effective KR preparations. The tumors were: transplantable sarcomas 37 and 180 in Swiss and A-mice; transplantable squamous cell carcinoma 119 in A-mice; transplantable and spontaneous mammary adenocarcinoma in C3H- and dba-mice.

Infections of mice with the various *T. cruzi* strains, *i.e.* active Chagas' disease, resulted in consistent inhibition of tumor growth, but produced very few tumor regressions and generally did not lengthen the life of infected cancerous mice beyond that of controls (*5, 6*). Tumor inhibition was sometimes accompanied by loss in body weight. These weight losses were closely correlated with the graded virulence of the several trypanosome strains.

The parasites were not "positively tumorotropic," as stated by the Russian investigators who found leishmanial stages in malignant growths to the exclusion of normal host tissues. Under our experimental conditions, cancer cells proper were only rarely parasitized. The infection was lightly present in the stroma of some tumors and was heavily concentrated in heart, liver, spleen, kidneys, small intestine, and skeletal muscle.

Cancerous mice infected with the always lethal W–BH strain died within 8–13 days of inoculation. Weight loss in these animals was considerable, and tumor growth was almost completely suppressed. When the infection in such mice was checked by drugging with the quinoline derivative, Bayer 7602, the previously inhibited tumors resumed their usual growth rate, and the hosts eventually died of cancer. From the available evidence, tumor inhibition by living *T. cruzi* does not appear to be a specific phenomenon caused by response to specific toxins, but can more adequately be attributed to competition for essential dietary factors and to general depletion of the host-system by the infection. Active Chagas' disease has, therefore, no clinical value in the treatment of malignant growths.

The killed trypanosome preparations of Roskin and Klyueva contained the "cancerolytic endotoxin," KR, which was claimed to have caused the complete regression of a variety of neoplasms in experimental rodents and in 13 out of 60 cancer patients. Follow-ups on the human cases treated are too brief for final appraisal, and evaluation is further confused by X-ray treatment in several instances. Duplicating as closely as possible the Russian techniques, we followed two general types of procedure:

(1) Cultures of *T. cruzi* (Brazil strain) at the height of growth were heat-killed by 30-min exposures to a temperature of 49° C ± 1°. This material was injected intraperitoneally into cancerous mice, or the tumor pieces were soaked in the preparation for 8 hrs prior to implanting. Injections were without effect, and the latter pretreatment did not reduce the number of "takes" or alter subsequent growth, as compared with an equal num-

[1] Our experiments, conducted under a grant from the National Cancer Institute, represent one aspect of a broad joint-institutional research program on chemotherapy of cancer, initiated and organized by Murray J. Shear. We are indebted for the technical assistance of Mrs. Jean Palmquist and Miss Elizabeth Brown. Various strains of *T. cruzi* were kindly supplied by Mrs. Eleanore Johnson Tobie (National Institute of Health), Cecil A. Hoare (Wellcome Laboratories of Tropical Medicine, London), and Malcolm H. Soule (University of Michigan).

ber of untreated controls (Table 1). Dosage was based on units, one unit being the equivalent of 1,000,000 killed trypanosomes.

KR stability by Roskin and Klyueva. The lysate was made under bacteriologically sterile conditions, and from 8 to 16 consecutive daily injections were given either

TABLE 1

Treatment	Total dosage (units)	Injections	Tumor	Mice (tests + controls)	% Growth of tests (contr. = 100)	Tumor Regressions		Deaths during expt.		Effect on test tumors
						Tests	Contr.	Tests	Contr.	
TESTS WITH HEAT-KILLED *T. cruzi* CULTURES:										
Heat-killed *T. cruzi* B strain from cultures	85–520	4–8 i.p.	Transpl. mammary	20 C3H—♀♀	97	0	0	1	0	None
	70	4 i.p.	Carc. 119	40 A—♂♂ + ♀♀	113	1	0	3	2	Ulcerated earlier
	1,050	15 i.p.	Spontan. mammary	96 dba— + C3H—♀♀	88	3	1	21	17	None
Test tumors soaked in above for 8 hrs before implantation			Carc. 119	20 A—♀♀	96	0	2	0	0	Ulcerated earlier
			Transpl. mammary	20 C3H—♀♀	128	0	0	0	1	None
Summary				196	104	4	3	25	20	
TESTS WITH LYSED *T. cruzi* CONCENTRATES:										
Lysed *T. cruzi* B strain cultures	600	16 s.c.	Carc. 119	20 A—♂♂ and ♀♀	144	0	0	2	6	None
Lysed *T. cruzi* W–BH strain from infected mice	300	8 s.c.	Carc. 119	20 A—♂♂ and ♀♀	120	0	0	9	1	Softening and necrosis
	600	11 s.c.	Spontan. mammary	20 dba—♀♀ and C3H—♀♀	68	1	1	Killed for tissue study		Hemorrhage, softening and necrosis
	850	14 s.c.	Carc. 119	20 A—♂♂ and ♀♀	117	0	0	1	0	None
Summary				80	112	1	1	12	7	
TESTS WITH "WHOLE CULTURE LYSATES" OF *T. cruzi*:										
Lysed *T. cruzi* S strain grown in NIH medium	1,120	28 11 s.c. 17 i.p.	Spontan. mammary	4 dba—♀♀		0	0	1	2	None
	"	"	Sarc. S–180 Sugiura	3 A—♀♀		1	1	1	2	"
	"	"	Carc. 119	13 A—♂♂ and ♀♀	92	0	0	3	3	"
Whole culture lysate *T. cruzi* S strain grown in NNN + Tyrode. Culture obt. from Malisoff	7	7	Carc. 119	28 A—♂♂ and ♀♀	Not measured	0	0	0	2	"
	"	"	Sarc. S–180 Sugiura	36 A—♀♀	"	0	0	18	18	"
	9	"	Sarc. T–180 Malisoff	44 Swiss—♀♀	119	10	12	12	10	"
Summary				128	105	11	13	35	37	

(2) Concentrations of *T. cruzi* centrifuged either from rich cultures of B-strain in NIH medium or from the plasma of mice bled at the height of infection with W–BH strain were lysed by the addition of neutral glass-distilled water and extracted at 1° C for 24 hrs prior to use. The age of lysate when injected ranged from 1 to 6 days, 10 days having been stated as the upper limit of subcutaneously or intraperitoneally. This treatment produced no tumor regressions, but did occasionally result in damage to both neoplastic and normal tissues, especially liver and kidney. Mortality was greater among the treated mice than in the untreated controls (Table 1).

Malisoff (*8*) modified the Russian technique by adding distilled water and 1: 10,000 Metaphen to whole cultures

of the S strain of *T. cruzi* grown on NNN medium with Tyrode overlay. These "whole culture lysates" were injected either intraperitoneally or subcutaneously and were quantitated to give the equivalent of about 1,000,000 lysed organisms/daily inoculum. We prepared such lysates from cultures received through Malisoff and from our own cultures of S strain. Among the various tumors tested (Table 1) were two lines of sarcoma 180; one obtained from K. Sugiura, of Memorial Hospital, and the other furnished and implanted in every case by the same technician who had supplied Malisoff with sarcoma T-180. The former tumor (Sugiura S-180) took and killed in all instances, while Malisoff's sarcoma T-180 regressed spontaneously in more than half of our controls.

Since the claims of Malisoff (*8*) are based on a total of only 43 treated mice and 15 controls, and since exact repetition of the T-180 experiments showed more than 50% tumor regressions in the untreated mice, all of Malisoff's results with sarcoma T-180 may be looked upon as spontaneous regressions. "Whole culture lysates" tested by us against spontaneous adenocarcinoma in 40 C3H- and dba-mice gave no sign of cancerolytic effect and did not prolong survival. On the contrary, deaths among the treated mice were more frequent than among the controls.

Malisoff's use of the term "chemical purification" gives the misleading impression of a fractionating technique followed in preparing the cancerolytic principle. Actually, whole culture lysates are crude preparations, and failure of other laboratories to reproduce potent KR should not be ascribed to degree of refinement in procedure. As for the work of Roskin and Klyueva, it is still too early for final judgment; but in view of the almost wholly negative outcome of our experiments (*5, 6*) and those of others (*1–3*), the elusive "endotoxin" of *T. cruzi* does not at present appear to hold out much promise for cancer therapy.

References

1. Belkin, M., Tobie, E. J., Kahler, J., and Shear, M. J. Personal communication on report presented at Atlantic City meeting of American Association for Cancer Research, March 1948.
2. Cohen, A. L., Borsook, H., and Dubnoff, J. W. *Proc. Soc. exp. Biol. Med.*, 1947, **66**, 440.
3. Engel, R. *Klin. Wschr.*, 1944, **23**, 127.
4. Hauschka, T. S. *J. Parasitol.*, 1947, **33**, 399.
5. Hauschka, T. S. *Proc. 4th int. Cancer Congr.*, 1947, in press.
6. Hauschka, T. S., Saxe, L. H., Jr., and Blair, M. *J. nat. Cancer Inst.*, 1947, **7**, 189.
7. Klyueva, N. G. *Amer. Rev. Soviet Med.*, 1947, **4**, 408.
8. Malisoff, W. M. *Science*, 1947, **106**, 591.

Three New Polymorphs of Zinc Sulfide

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Three new polymorphs of ZnS have been recognized by the writers. All are related structurally to wurtzite, but differ in that they represent stacking sequences of hexagonal closest-packing of higher periodicity than that of the basic wurtzite structure. The new polymorphs correspond to 4H, 6H and 15R in the notation of Ramsdell (*1*) and are isostructural with the corresponding polymorphs of silicon carbide. Table 1 shows the crystallographic properties, data for ordinary wurtzite (2H) being given for comparison.

TABLE 1

	Wurtzite–2H	Wurtzite–4H	Wurtzite–6H	Wurtzite–15R
a_0	3.811 A	3.806	3.813	3.822
c_0	6.234	12.44	18.69	46.79
$a_0 : c_0$	1 : 1.6358	1 : 3.268	1 : 4.902	1 : 12.242
Cell contents	Zn_2S_2	Zn_4S_4	Zn_6S_6	$Zn_{15}S_{15}$
Space group	C6mc	C6mc	C6mc	R3m

The crystals of the new polymorphs are steep pyramidal in habit and are doubly terminated with the basal pinacoid present at the analogous pole only. Parallel intergrowths of 15R with 4H and of 15R with 6H were observed, the surfaces of juncture being uneven and approximately vertical in position. In physical properties the several polymorphs are identical with wurtzite-2H. All have a distinct cleavage on $\{11\bar{2}0\}$ and traces of cleavage on {0001}. The specific gravity, determined by a microbalance on a 20-mg sample composed largely of 4H, was 4.09, in close agreement with the calculated value of 4.121 for 4H. Sufficient material for a quantitative chemical analysis is not at hand, but the material probably contains at least several per cent of divalent iron in isomorphous substitution for zinc.

The crystals occur in shrinkage cracks in clay-ironstone (siderite) concretions embedded in a carbonaceous black shale of the lower Conemaugh formation at numerous localities in western Pennsylvania and eastern Ohio. Associated minerals are barite, sphalerite, chalcopyrite, pyrite, and calcite. The concretions and minerals therein apparently formed at essentially ordinary conditions of temperature and pressure during the diagenesis of the sediment. All three of the polymorphs occur side by side in rudely radial aggregates of single crystals, indicating simultaneous crystallization. Sphalerite is later formed than the polymorphs, and wurtzite-2H does not occur in the assemblage.

[1] We wish to express our appreciation to David M. Seaman, of the Carnegie Museum, Pittsburgh, and Howard Hamilton, of Vandegrift, Pennsylvania, who found the original specimens and generously offered them for study.

Reference

1. Ramsdell, L. S. *Amer. Min.*, 1947, **32**, 64.