TABLE II. Correlation of Microbiological and Radioactivity in the Butanol Extract.

Group*	No. of rats	A = Radioactivity avg of group c.p.m. × 10^{-3} per sample	B = Microbiological activity group avg expressed in γ/sample B_{12}	A/B
W	6	2	.9	2.2
X	3	6	2.8	2.1
Y	2	5	2.0	2.5
U	3	9	3.4	2.6

* See Table I for composition of group diets.

TABLE III. Fecal Weights of Rats on Diets with or Without Aureomycin.

Group*	No. of fecal samples	Sex	Avg wt of feces in g/2-day collection
W	26	F	3.1 ± .23
X	26	F	4.2 ± .32
Y	6	M	5.3 ± .3
U	11	M	6.3 ± .4

* See Table I for composition of group diets.

reached only by actual isolation.

Another observation which is worth recording deals with the relative fecal matters collected from different groups of rats. The fecal weights are tabulated in Table III. Although all animals had approximately the same diet allowance, the feces of those in the aureomycin groups (X, Y, and U) weighed more than those in the control group W. Furthermore, this difference in the fecal weight manifested itself not only during any single period but was sustained during the entire experiment.

Discussion. The fact that the addition of antibiotics in appropriate amounts can improve the nutritional status of the host can be accounted for on the assumption that the bactericidal agent selectively destroys certain microorganisms, (a) which will otherwise compete with the host for accessory factors synthesized by another group of organisms, or (b) which produce toxins that might interfere with the utilization of nutrients. The data presented in this paper provide direct evidence that the addition of aureomycin results in an increase in the vit. B_{12} content of the fecal matter. Our experiments do not exclude the possibility that the fecal content of other accessory factors might also have been increased as a result of aureomycin feeding. Such an hypothesis could explain some of the findings of Jukes *et al.*, who demonstrated some beneficial effects to growing animals after supplementation of aureomycin in diets with adequate supply of vit. B_{12}. It is also important to point out that, in this series of experiments, only aureomycin was used, although other antibacterial agents might be expected to produce similar effects.

Summary. By the incorporation of Co^{60} into the diet, it could be shown that aureomycin causes an increase in the activity, both radioactive and microbiological, of the vit. B_{12} content of the feces. The fecal B_{12} content could be increased by increasing the amount of Co^{60} used. The experimental findings can be explained by the effect of the antibiotic on the intestinal bacterial flora.

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Filtrates of Fresh Tumor Injected Prior to Transplantation of the Homologous Tumor.* (18730)

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Anaerobic refrigeration in the deep freeze (0-24°F) for 10 days or longer (until the cells are no longer viable) or lyophilization for a similar period of Brown-Pearce tumor tissue(1,2), Bashford Ca 63(3-5), 15091a (6-8) or Eo771(6-9) tumor tissue results in the presence of specific(5-9) substances which,

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TABLE I. Preparation of and Injection Intervals of Filtrates of Fresh Untreated Emulsions of the Brown-Pearce Tumor.

Exp.	Tumor type	Filtrate site inoc.	Tumor int. days	Transplant site inoc.	Experimental N	I	D	T	Controls N	I	D	T
1	BVF	LT	14	RT	5	1	0	6	1	3	2	6
2	BVF	LT	14	RT	0	1	3	4*	2	2	2	6
3	BNF	SC	13	SC	3	2	0	5	4	1	0	5
4	SF	SC	20	SC	3	1	1	5*	3	0	1	4*
5	SF	SC	23	SC	4	1	0	5	1	0	2	3*
6	SF	SC	21	SC	3	1	1	5	1	2	1	4*
				Totals	18	7	5	30	12	8	8	28

Exp.—experiment No.; BVF—Berkefeld "V" filtrate; BNF—Berkefeld "N" filtrate; SF—Seitz filtrate; LT—left testis; RT—right testis; SC—subcutaneous; int. days—time interval between inoculation of filtrate and tumor transplantation; N—animals without primary or metastatic tumor at necropsy; I—animals alive but with primary or metastatic tumor at necropsy; D—animals dying from growth and metastases of the Brown-Pearce tumor; *—animals dying from intercurrent disease under 21 days after tumor transplantation were omitted from the tabulation (Table II).

when injected into animals prior to transplantation of the homologous tumor, cause the transplantation to be more successful in homologous(9) and in heterologous(1,6-8) hosts, and the metastases and mortality to be increased(1,2). The Brown-Pearce "autolyzed" material (called XYZ) was filtrable through a Berkefeld "V" filter(10).

Attempts were then made to see whether the same effect could be induced by injecting a cell-free filtrate of fresh tumor tissue 10-14 days before tumor transplantation. Berkefeld "V"(11) and Seitz(12,13) filtrates of fresh Brown-Pearce tumor failed to induce a state of susceptibility in the injected animals. In fact, the reverse effect was observed. Since marked physical and perhaps chemical disintegration of the cells was induced by the repeated freezing and thawing before filtration employed by Raab and by Malluche(12,13), it seemed worthwhile to do additional experiments with the Brown-Pearce tumor using filtrates of fresh tumor tissue which had not been killed and disintegrated by rapid freezing and thawing.

Materials and methods. In 6 experiments 66 rabbits were injected intratesticularly or subcutaneously with an emulsion of Brown-Pearce tumor tissue. The methods of preparation, inoculation, postmortem examination and analysis have been described(1,2). There were 33 control and 33 experimental animals matched as closely as possible as to age, breed, weight, etc. (Table I). The animals were chosen on receipt from dealer on the basis of apparent health yet 6 experimental and 5 control animals died of intercurrent affection during the 3 to 5 months which the experiments lasted, 8 of these shortly after reception. No further disease was noted at postmortem examination. In 2 experiments 0.3 cc of a Berkefeld "V" filtrate of fresh tumor tissue (1-4 emulsion in sterile pneumococcus broth pH 7.8) was injected into the left testis and 14 days later 0.3 cc of a saline emulsion of Brown-Pearce tumor transplanted into the right testis. In a third experiment a Berkefeld "N" filtrate of fresh Brown-

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2. Casey, Albert E., *Cancer Research,* 1941, v1, 134.
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10. Casey, Albert E., *Am. J. Cancer,* 1936, v26, 276.
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12. Raab, W., *Z. f. Krebsforsch,* 1937, v46, 343.
13. Malluche, Harold, *Beitr. z. klin. Chir.,* 1938, v167, 343.

Pearce tumor was injected subcutaneously 13 days before subcutaneous transplantation of the tumor. In 3 additional experiments 0.3 cc of a Seitz filtrate of fresh tumor tissue (Brown-Pearce) was injected once 20-23 days prior to subcutaneous transplantation of the Brown-Pearce tumor.

Results. The results are summarized in Table II. The percentage of deaths, the average number of metastatic foci and the total tumor per animal were less (statistically insignificant) and the number of animals which failed to grow the tumor more (statistically insignificant) among the groups of animals having pre-transplantation treatment with the filtrates. It was concluded therefore that the XYZ factor for the Brown-Pearce tumor either was not present or was biologically inactive in filtrates (Berkefeld V, N and Seitz) of fresh emulsion of the Brown-Pearce tumor.

Discussion. Domagk and Hackmann(14) and Dittmar(15) injected filtrates of fresh Ehrlich mouse carcinoma or sarcoma prior to tumor transplantation, and recorded inhibitory results similar to ours with the Brown-Pearce tumor. However, no XYZ factor has been demonstrated for this tumor. Recently Kaliss, Jonas and Avnet(16) reported in an experiment employing 21 animals that a filtrate of fresh mouse mammary tumor 15091a failed to sensitize a heterologous strain of mice to the homologous (15091a) tumor, whereas the lyophilized tumor tissue was quite potent (6-8) in this respect.†

The complete experience of Raab, Malluche and ourselves with the Brown-Pearce tumor, of Dittmar with the Ehrlich mouse sarcoma and of Kaliss, Jonas, and Avnet with 15091a was summarized (Table III). In each experiment by each of the authors the fresh filtrate-injected animals grew fewer tumors than the controls (not statistically significant). This is in contrast to the statistically significant and widespread tumor growth and metastases following a preliminary injection of a Berkefeld "V" filtrate of homologous tumor tissue which has been kept frozen (the tumor tissue) for 10-21 days(10). Since the Brown-Pearce and other XYZ or lyophilization factors seem specific and always present in the homologous refrigerated tumor tissue they must be "bound" or in some combination in fresh tumor,‡ and must be activated

14. Domagk, G. and Hackmann, Ch., *Z. f. Krebsforsch,* 1935, v42, 192.

15. Dittmar, Carl, *Z. f. Krebsforsch,* 1936, v45, 109.

16. Kaliss, Nathan, Jonas, Gordon and Avnet, Norman L., *Cancer Research,* 1950, v10, 228.

† The specific lyophilization or XYZ factor of mouse carcinoma 15091a(6,7,8,9) was present but in less active form in fresh(16) unfiltered tumor tissue and in its sedimented cells (28 of 63 C57 mice or 44% grew this Line A tumor; 0% of the controls) and was abundant in lyophilized(8) tumor tissue (141 of 184 C57 mice or 77% grew the Line A tumor; 0% of 202 controls). The factor was not demonstrated in the filtrates of fresh tumor (0 of 21 or 0%) but was suggestively present in fresh unfiltered normal liver, spleen and kidney of Line A mice (13 of 82 C57 mice or 16% grew the Line A tumor; 0% of controls) and definitely present in lyophilized normal liver, spleen and kidney of Line A mice (47 of 105 C57 mice, or 45%, grew the Line A tumor; 0% of controls). The question arises as to whether the specific 15091a factor is a silent virus in the sense of the "milk agent" widely distributed in an inbred line of mice(17), or a somatic host factor similarly placed. The specific XYZ factor in C57 black mammary carcinoma Eo771 did not seem to be a somatic host factor since it could not be demonstrated in C57 black mouse mammary carcinomata 241-5, 241-16, 755, or 946 or in several other neoplasia of C57 black origin (9,18). Furthermore, the XYZ phenomenon seems to be a technic by means of which the Eo771 XYZ factor can be readily eliminated from this tumor(18) and the specific factor, therefore, is not somatic.

17. Barnum, Cyrus P., Ball, Z. B., and Bittner, John J., *Cancer Research,* 1947, v7, 522.

18. Casey, Albert E., *Acta, Fifth International Cancer Congress,* Paris, France, July 17, 1950; Annual Rep. to Com. on Growth, Nat. Research Council, acting for the American Cancer Society.

‡ However, an accelerating factor was demonstrated in the unfiltered supernatant fluid which had been prepared by centrifugation repeatedly of a saline extract of fresh (unfrozen) mouse mammary carcinoma Z8352. This tumor, which originated in a female C3H mouse possessing the milk agent, is maintained by transplantation in ZBC mice not possessing the milk agent(22).

22. Shear, Howard H., in press.

TABLE II. Results of the Prior Injection of Filtrates of Fresh Tumor on the Course of the Brown-Pearce Rabbit Tumor.

A. Average number of metastases and average volume of primary and metastatic tumor.

	Per animal inoculated						Per animal with tumor					
	Filtrates			Controls			Filtrates			Controls		
Sites	R, #	MF, #	TT, cc	R, #	MF, #	TT, cc	R, #	MF, #	TT, cc	R, #	MF, #	TT cc
LT–RT	10	6.2	34	12	6.8	52	5	12.4	68	9	9.1	70
SC–SC	20	1.8	30	16	3.9	64	7	5.0	84	7	8.9	146
Total	30	3.2	31	28	5.2	59	12	8.8	78	16	9.1	103

B. Animals dying from tumor growth versus animals completely negative.

	Negative				Died from tumor				Alive with tumor				Total	
	Filtrates		Controls		Filtrates		Controls		Filtrates		Controls			
Sites	#	%	#	%	#	%	#	%	#	%	#		F	C
LT–RT	5	50	3	25	3	30	4	33	2		5		10	12
SC–SC	13	65	9	56	2	10	4	25	5		3		20	16
Total	18	60	12	43	5	17	8	29	7		8		30	28

R—rabbits; MF—avg No. of metastatic foci per animal; TT—avg volume of primary and metastatic tumor per animal; filtrates—animals treated with filtrates of fresh Brown-Pearce tumor 13-23 days before transplantation of the Brown-Pearce tumor; controls—animals not so treated; #—No.; cc—cubic centimeters, measured by water displacement; LT—left testis, site of filtrate injection; RT—right testis, site of tumor transplantation; F—filtrates; c—controls; A.W.T.—indeterminate (alive with tumor at the termination of the experiment); none of the differences between filtrate and control series were statistically significant.

TABLE III. Summary of Results by Various Authors of the Injection of Filtrates of Fresh Tumor Prior to Tumor Transplantation.

		Animals			Negative				Grew tumor			
					Filtrate		Controls		Filtrate		Controls	
Author	Tumor	F #	C #	Total #	#	%	#	%	#	%	#	%
1 LT–RT	BP	10	12	22	5	50	3	25	5	50	9	75
1 SC–SC	BP	20	16	36	13	65	9	56	7	35	7	44
2 IT–IT	BP	46	120	184	16	35	34	28	30	65	86	72
2 SC–IT	BP	18			7	39			11	61		
3 IV–IT	BP	29	12	41	3	10	1	8	26	90	11	92
Subtotal	BP	123	160	283	44	36	47	29	79	64	113	71
4 SC–SC	EMS	159	102	261	43	27	17	17	116	73	85	83
5 SC–SC	15091a	11	10	21	11	100	10	100	0	0	0	0
Total		293	273	565	98	33	74	27	195	67	198	73

F—filtrate-injected animals; C—control animals; Total—total animals; 1—see Table II; 2—Malluche(13); 3—Raab(12); 4—Dittmar(15); 5—Kaliss, Jonas and Avnet(16); LT-RT—filtrate injected into left and tumor into right testis; SC—subcutaneously; IT—intratesticularly; IV—intravenously; BP—Brown-Pearce rabbit tumor; EMS—Ehrlich mouse sarcoma; 15091a—mammary carcinoma 15091a of Line A origin.

by slow autolysis in the cold, or are attached to or parts of a macromolecule which is not filtrable through Berkefeld or Seitz filters. The slight inhibitory influence noted in filtrates of fresh tumor tissue could be of the nature described by Gorer(19), by Kaliss and Jay(20), and by Murphy, Helmer, Claude and Sturm(21) and others. It would seem to bear no relation to the specific XYZ or lyophilization factors found in Brown-Pearce, Bashford 63, Eo771 or 15091a tumors.

Summary. In 6 experiments involving 66 rabbits, Berkefeld "V", "N" and Seitz filtrates of fresh Brown-Pearce tumor tissue did not contain evidence of the specific XYZ or lyophilization factor, although the factor has been uniformly present in Berkefeld "V" filtrates of tumor tissue after prolonged freezing.

19. Gorer, P. A., *Cancer Research,* 1947, v7, 634; *J. Path. and Bact.,* 1937, v44, 691; *ibid.,* 1938, v47, 231; *ibid.,* 1942, v54, 51.

20. Kaliss, Nathan, and Jay, George E., Jr., *Cancer Research,* 1950, v10, 227.

21. Murphy, J. P., Helmer, O. M., Claude, A., and Sturm, E., *Science,* 1931, v73, 268.

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