the antibody response was decreased in the pantothenic acid-deficient rats of all groups. In no instance, did the titer of a deficient rat equal that of its "paired-weighed" control. The deleterious effect of a mild pantothenic acid deficiency is evidenced by the decreased titers of the animals in Group I which were on experiment for only 3 weeks prior to immunization. This observation lends no support to the explanation offered by Stoerk for the discrepancy in the findings of the two laboratories. The comparatively high antibody content of the "paired-weighed" controls is further evidence against the role of inanition in the decreased antibody response of pantothenic acid-deficient rats.

In the course of further studies on the mechanism of action of pantothenic acid we have determined the antibody response in 44 pantothenic acid-deficient rats and 29 suitable controls. The results obtained are in excellent agreement with those recorded in this as well as in a previous paper.[1] All of the evidence to date firmly implicates pantothenic acid as a vital factor in the antibody response to the antigenic stimulus of human erythrocytes.

The thymus weights of the pantothenic acid-deficient rats were lower than those of the control group (Table I). These findings are in agreement with those made by Stoerk *et al.*[2]

Summary. (1) Hemagglutinin production in response to inoculation with human erythrocytes has been investigated in rats fed a pantothenic acid-deficient diet for 3, 5, and 7 weeks. "Paired-weighed" animals served as inanition controls.

(2) Marked impairment of antibody response was observed in all pantothenic acid-deficient groups.

Received July 27, 1949. P.S.E.B.M., 1949, **72**.

Selective XYZ Factor in C57 Black Mammary Carcinoma Eo771. (17339)

ALBERT E. CASEY, GORDON L. ROSS, AND R. REVAE LANGSTON

*From the Departments of Pathology of the Baptist Hospital of Alabama, and the Medical College of Alabama, Birmingham, Ala.**

The XYZ phenomenon may be defined as the increased incidence and more rapid increment of local and metastatic transplanted tumors following the prior injection in the same or distant sites of an extract from the homologous tumor.[1-4] The factor in the Brown-Pearce rabbit tumor responsible for the phenomenon is selective, filtrable (Berkefeld "V") and thermolabile (56°C);[5-7] that in Bashford mouse mammary carcinoma 63[8,9] is likewise highly selective or specific.[10-12]

* The work was aided by grants from the American Cancer Society and the Damon Runyon Fund through the Committee on Growth of the National Research Council. The assistance of Joe Norman, Bruce A. Elrod, Robert C. Rea, Erma L. Salter, Gertrude Hampton, Irene Hartsfield, Betty Baker, Russell Laster, and Ward Talley of the laboratory staff of the Baptist Hospital in the care of the animals, recording of data and preparation of histologic material is acknowledged with thanks.

1 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 816.

2 Casey, Albert E., *Am. J. Cancer*, 1934, **21**, 760.

3 Casey, Albert E., *Am. J. Cancer*, 1934, **21**, 776.

4 Casey, Albert E., *Cancer Research*, 1941, **1**, 134.

5 Casey, Albert E., *Am. J. Cancer*, 1936, **26**, 276.

6 Casey, Albert E., and Moragues-Gonzales, Vincent, *Am. J. Cancer*, 1940, **38**, 59.

7 Casey, Albert E., Meyers, Lucille, and Drysdale, George R., 1948, **69**, 570.

8 Haaland, M., *Proc. Roy. Soc. London*, 1910, **82**, 293; *Lancet*, 1910, **1**, 787.

9 Leitch, A., *Lancet*, 1910, **1**, 991.

10 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 674.

11 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1025.

12 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 663.

Brown-Pearce and Bashford tumor tissues aseptically removed from the host, frozen immediately and kept frozen at 0-24°F until the cells are no longer viable uniformly contain the respective XYZ factors in great quantity; cultures for bacteria have been sterile[8,9,13] and the Brown-Pearce tumor tissue has been free from Virus III, rabbit pox and other known viruses.[14] No reaction appears at the site of single or repeated injections of the XYZ materials. Similarly prepared extracts of normal tissues or of other tumors even in the same species when injected prior to transplantation of Bashford 63 or the Brown-Pearce tumor have not elicited the XYZ phenomenon.[5,13,15]

Recently Snell, Cloudman, Failor, and Douglass,[16] and Snell, Cloudman, and Woodworth[17] have reported similarly planned experiments using inbred strains of mice. Their data indicate to us enhancement of homologous tumor growth entirely acceptable to the above definition of the XYZ phenomenon by factors present in C57 Black mouse mammary carcinoma Eo771 and Line A mouse mammary carcinoma 15091a. Their technic consisted in freezing and storing the tumor tissue with lyophilization as an added routine procedure. Lyophilization has been found to be an effective method of storing the XYZ factor from the Brown-Pearce tumor.[18] Desiccation after freezing of the Brown-Pearce tumor by the methods current in 1930, resulted in some loss in the potency of the XYZ factor,[1] and the more modern lyophilization methods such as those used by Snell, Cloudman, and Woodworth are a great improvement in the study of the XYZ factors.

The present communication reports experiments by us with Eo771 and correlates our results with those previously obtained by Snell, Cloudman, and Woodworth.

Material and methods. The animals were 250 C57 black mice received from Carworth Farms, New City, New York over a period of one year in batches of 40 (animals of each batch of same sex). On receipt the animals were placed in metal self-feeder mouse boxes 10 to a box, and alternate boxes marked "control" and "experimental" respectively. The animals were allowed from 1-4 months to mature and become adjusted to the new environment. The animal quarters were air conditioned throughout the year, temperature set at 70°F and humidity at "ideal." Some 8 experimental and 9 control mice died of intercurrent disease over an average 6-8 months observation period (loss 6.8%) and usually before experiments were initiated. Since the diagnosis in each was checked by necropsy and histologic examination these 15 mice were eliminated from the series. In no instance did injection of XYZ material lead to local infection or abscess. Except in 2 experiments (40 mice) no evidence of infection appeared at the sites of tumor transplantation. In the 2 experiments above mentioned abscesses (4-5 mm in diameter) appeared at the site of tumor transplantation in about 30-40% of control as well as experimental animals. In each instance transfer had been made from a large growth having considerable necrosis. The number of takes in the 2 experiments was less than normal, although good tumors eventually grew from most of the animals. No animal died because of the intradermal abscesses, and all lesions were healed for the most part by 15 days.

There were 12 separate experiments with equal numbers in control and experimental series with one exception in which there were 20 controls instead of the usual 10 (Table I). Measurements of the tumors in 3 dimensions in the controls and experimental animals were made concomitantly at intervals after inoculation of the tumor tissue. Although the trends remained uniform from the first meas-

[13] Drysdale, George R., and Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 306.

[14] Pearce, Louise, and Casey, Albert E., 1933 (unpublished experiments, The Rockefeller Institute for Medical Research).

[15] Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 731.

[16] Snell, George D., Cloudman, Arthur M., Failor, E., and Douglass, P., *J. Nat. Cancer Inst.*, 1946, **6**, 303.

[17] Snell, George D., Cloudman, Arthur M., Woodworth, Eliz., *Cancer Research*, 1948, **8**, 429.

[18] Drysdale, George R., Ross, Gordon L., Skipper, Howard, Edwards, P. C., and Casey, Albert E., unpublished experiments.

TABLE I.
Experiments with XYZ Factor from Mammary Carcinoma Eo771, Using C57 Black Mice.

Injection of XYZ factor				Data on tumor transplantation and growth						
Origin of XYZ	Stored Days	Stored Temp.	Days prior	Tumor and its origin	Host mice	Total mice	Grew tumor	Σ_x	M_x	Σ_{x2}
				A. Heterologous XYZ and Heterologous Host.						
Eo771 57bl	45	0°F	34	15091a A	57bl	10	0	0.00	0.00	0.0
Controls				15091a A	57bl	9	0	0.00	0.00	0.0
				B. Heterologous XYZ and Homologous Host.						
Ba. Ca. C3H	337	0°F	16	Eo771 57bl	57bl	10	9	23.70	2.37	108.5
Controls						10	10	26.90	2.69	102.2
Eo771 57bl	45	0°F	43	241-5 57bl	57bl	10	5			
Eo771 57bl	45	0°F	43	241-5 57bl	57bl	8	2	15.42	0.86	56.5
Controls				241-5 57bl	57bl	8	6			
Controls				241-5 57bl	57bl	10	3	57.31	3.18	469.0
E0771 57bl	45	0°F	50	241-16 57bl	57bl	8	4	10.14	1.27	85.0
Controls				241-16 57bl	57bl	20	12	36.52	1.83	216.8
241-5 57bl	68	0°F	10	Eo771 57bl	57bl	9	7			
241-5 57bl	68	0°F	10	Eo771 57bl	57bl	9	8	65.36	4.36	450.3
Controls				Eo771 57bl	57bl	10	6			
Controls				Eo771 57bl	57bl	10	10	74.64	3.73	459.5
241-5 57bl	68	0°F	34	Eo771 57bl	57bl	9	5	10.38	1.15	23.8
Controls				Eo771 57bl	57bl	9	8	17.18	1.91	58.4
Summary XYZ injected animals						63	40	124.94	1.98	723.3
Summary controls						77	55	212.65	2.76	892.0
				C. Homologous XYZ and Homologous Host.						
Eo771 57bl	45	0°F	25	Eo771 57bl	57bl	9	9			
Eo771 57bl	45	0°F	25	Eo771 57bl	57bl	10	10	135.37	7.12	1352 0
Controls				Eo771 57bl	57bl	10	6			
Controls				Eo771 57bl	57bl	8	8	52.86	2.94	302.4
Eo771 57bl	28	0°F	18	Eo771 57bl	57bl	10	10	63.44	6.34	472.5
Eo771 57bl	28	0°F	19	Eo771 57bl	57bl	10	9	4.98	0.50	3.9
Controls				Eo771 57bl	57bl	9	7	4.25	0.47	3.5
Controls				Eo771 57bl	57bl	8	8	25.72	3.22	110.1
Summary XYZ injected animals						39	38	203.79	5.23	1828
Summary controls						35	29	82.83	2.37	416

urement in each of the 12 experiments, the measurements used were the last series taken before the first animals of either test or control groups died from the tumor. The tumors employed were Eo771, 241-5, and 241-16, mouse mammary carcinomata, of C57 black origin; Barrett mammary carcinoma of C3H origin; and 15091a mammary carcinoma of Line A origin.† In each experiment Eo771 was used either as the source of the XYZ material or as the source of the tumor tissue, or both.

† The mouse tumors were obtained through the courtesy of various persons: Eo771 and 15091a from Dr. George D. Snell of Bar Harbor, Maine, 241-5 and 241-16 from Dr. Howard B. Andervont, Dr. Thelma Dunn and Dr. Harold L. Stewart, and the Barrett carcinoma from Dr. M. K. Barrett, and C57 black mammary carcinoma 755 from Dr. Edwin D. Murphy, all of Bethesda, Md.

Since Eo771, 241-5 and 241-16 originated in C57 black mice, such mice were considered "homologous hosts" when any of the 3 tumors was transplanted into them. On the other hand 15091a (of Line A origin) transplanted in C57 black mice, was said to have been inoculated into a heterologous host.

The XYZ material, if from a different tumor than that transplanted, was said to be heterologous, even though originating in the same anatomic site in the same inbred strain (*i.e.* 241-5, 241-16 and Eo771 in C57 black mice). Experiments with another mammary carcinoma No. 755 originating also in C57 black mice[19] are now in progress.

The XYZ material was prepared by placing fresh aseptically removed tumor tissue in

[19] Kaplan, Henry S., and Murphy, Edwin D., *J. Nat. Cancer Inst.*, 1949, **9**, 407.

the deep freeze chamber at 0°F and keeping it there for 28-337 days. In each instance a portion of the tumor tissue to be frozen was tested for viability by routine transfer. Each tumor for XYZ or for transplant was verified by histologic examination, using the blind check method for tumor identification. 241-5 and 241-16 were quite similar histologically and cage records were necessary to differentiate between them.

Since the Brown-Pearce and Bashford 63 XYZ factors withstand storage in the cold for at least a year and since a single injection leaves prolonged hypersusceptibility to the same tumor, some latitude was exercised in the storage (45-337 days) and interval before reinoculation (10-50 days) in the Eo771 experiments (Table I). The measurements of the transplanted tumor were made on the same day for both control and test mice, using calipers. The "volume" was the product of the caliper diameters in 3 dimensions. Although this introduced a considerable error when checked by weight of the tumors it gave a uniform value for both test and control animals.

The XYZ material and the tumor tissue for transplantation were prepared for inoculation by mincing and grinding in a mortar and diluting with normal saline (1 part tumor and 3 parts saline). In the last 2 experiments (Table I) Penicillin G sodium (30,000 units cc) buffered with sodium citrate (Lederle) was used as the diluent in the place of normal saline. 0.1 to 0.15 cc of the emulsion of the frozen XYZ or of the fresh tumor tissue as the case might be, was inoculated subcutaneously into the left groin.

A necropsy with histologic examination of lungs, heart, liver, and primary inoculation site and other selected tissue was made on each of the 250 mice. Because of the rapid growth of the tumors experiments in which the animals were transplanted with Eo771 were terminated at 15-30 days, whereas experiments with 241-5 and 241-16 were not terminated before 40-70 days depending on when the first animal died.

Results. The results are presented in tabular form under 3 categories, namely: A. Heterologous XYZ and heterologous host; B. Heterologous XYZ and homologous host; C. Homologous XYZ and homologous host (Table I). In no instance did a heterologous XYZ material produce an XYZ effect in either a heterologous or in a homologous host (A and B, Table I). The summarized material indicates that 40 of the 63 heterologous XYZ injected animals (63%) had progressively enlarging tumor growths with an average size of 1.98 cc whereas 55 of the 77 control mice (71%) had progressively enlarging growths which averaged 2.76 cc (the difference $= 0.78 \pm 0.42$ cc; $t = 1.9$; $n = 138$; $P = 0.06$, not significant). This difference would indicate that the heterologous XYZ material produced a statistically insignificant inhibition of tumor growth. Likewise there was an insignificant inhibition of the incidence of transplanted tumors among the XYZ animals ($X^2 = 0.99$; $n = 1$; $P = 0.4$, not significant). The mean tumor size when the data for the Barrett carcinoma experiment was eliminated was 2.77 cc for 67 control mice as compared with 1.91 cc for 53 heterologous XYZ injected mice. The difference 0.86 ± 0.45 cc is suggestive of an inhibition.

In contrast to the above data the 39 animals injected with homologous XYZ into an homologous host had 38 progressively enlarging primary tumors (97%) as compared, with 29 progressively enlarging tumors among the 35 control mice in the 4 experiments (83%). The tumors averaged among the 39 XYZ injected animals 5.23 cc as compared with 2.37 cc among the 35 controls. The difference 2.86 ± 0.84 cc is statistically significant ($t = 3.4$; $n = 72$; $P = 0.01$—significant).

Discussion. In order to provide a basis for comparison with present results, the data of Snell, Cloudman, and Woodworth[17] pertaining to Eo771 has been abstracted and rearranged in the manner of the present experiments (Table II). This data includes observations on 213 inbred mice in which either Eo771 XYZ or Eo771 tumor transplants or both were used. There were three experiments with heterologous XYZ in heterologous hosts. The 26 XYZ injected animals had 17 progressively enlarging primary tumors (65%) as compared with 28 progressively en-

TABLE II.

Other Experiments with C57 Black Mouse Mammary Carcinoma Eo771. (Data abstracted and rearranged from the paper by Snell, Cloudman, and Woodworth[17] to correspond with the format in Table I).

Injection of XYZ factor				Data on tumor transplantation and growth				
Origin of XYZ	Stored Days	Stored Temp.	Days prior	Tumor and its origin	Host mice	Total mice	Died, tumor	Maximum mean tumor size, sqcm
			A. Heterologous XYZ and heterologous host.					
Eo771 57bl	?	lyoph.	8-12	C1498 57bl	57ln	6	4	5.53
Eo771 57bl	?	"	8-12	C1498 57bl	57ln	10	3	0.30
L946 57bl	?	"	8-12	Eo771 57bl	57ln	10	10	9.82
Controls				C1498 57bl	57ln	10	0	0.65
"				C1498 57bl	57ln	10	6	1.94
"				C1498 57bl	57ln	10	7	?
"				C1498 57bl	57ln	10	7	?
"				Eo771 57bl	57ln	8	8	12.99
Summary XYZ injected animals						26	17	5.17
Summary controls						48	28	4.64
			B. Heterologous XYZ and homologous host (no experiments reported).					
			C. Homologous XYZ and homologous host (no experiments reported).					
			D. Homologous XYZ and heterologous host.					
Eo771 57bl	?	lyoph.	8-12	Eo771 57bl	57ln	10	8	3.26
Eo771 57bl	?	"	8-12	Eo771 57bl	57bred	10	0	0.25
Eo771 57bl	?	"	15	Eo771 57bl	C58	15	15	4.97
Eo771 57bl	?	"	8-12	Eo771 57bl	C58	10	7	2.31
Eo771 57bl	?	"	8-12	Eo771 57bl	BAlbC	12	3	0.17
Eo771 57bl	?	"	8-12	Eo771 57bl	BAlbC	12	8	5.94
Eo771 57bl	?	"	8-12	Eo771 57bl	BAlbC	9	7	3.90
Controls				Eo771 57bl	57ln	5	3	4.25
"				Eo771 57bl	57bred	10	0	0.13
"				Eo771 57bl	C58	14	2	0.24
"				Eo771 57bl	C58	10	0	0.13
"				Eo771 57bl	BAlbC	12	0	0.42
"				Eo771 57bl	BAlbC	10	0	0.44
Summary XYZ injected animals						78	48	3.09
Summary controls						61	5	0.60

larging tumors among 48 controls (58%). This difference in incidence was not significant ($X^2 = 0.356$; n = 1; P = 0.6, not significant). The average size of the tumors in the 26 heterologous XYZ injected mice was 5.17 sq cm as compared with 4.64 sq cm among the 48 controls. The difference 0.53 ± 0.47 sq cm was not statistically significant.

When the data for heterologous XYZ injected animals of Tables I and II with their respective controls were combined there were 57 progressively enlarging tumors among 99 heterologous XYZ animals (58%), and 83 progressively enlarging tumors among 134 controls (62%). The difference was not statistically significant. The mean size of the tumors in the two groups was almost identical and the data of Snell, Cloudman and Woodworth and ours are in entire agreement. To us the data indicates that heterologous XYZ material has no appreciable effect on transplanted tumor growth in either homologous or heterologous hosts.

In 7 experiments 48 of the 78 mice injected with homologous XYZ material by Snell, Cloudman, and Woodworth had progressively enlarging tumors (62%) as compared with 5 tumors among 61 control mice (8%). This difference was statistically significant ($X^2 = 40.4$; n = 1; P = 0.0001—significant). The tumors among the 78 experimental mice averaged 3.09 sq cm and among 61 controls 0.60 sq cm. The difference 2.49 ± 0.34 sq cm was statistically significant (t = 4.3; P = 0.0001—significant). The key to the puzzle would seem to be the use of homologous XYZ material, and with such material heterologous inbred hosts were not always resistant to tumors of foreign strain origin.

In the experiments summarized in Tables I and II cross XYZ reactions were studied among 5 neoplasms of C57 black origin, and

the reactions in a sixth are now being tested (mammary carcinoma 755). Four of the 6 are mammary carcinomata of C57 black origin. (15091 of Line A origin, and Barrett carcinoma of C3H origin are also mammary carcinomata). If the XYZ material be of cellular origin it must be a permanent chemical mutation not present in the other mammary tissues and tumors of the same inbred strain. It must be perpetuated ad infinitum in the specific cells, but in no other and must exert a powerful influence on the continued transplantabiltiy and growth of the specific cells.

If the XYZ material be an inert contaminating virus[20] (a possibility which was considered early in the work)[14] it might conceivably act as follows: The injected XYZ material immunizes the host to the virus and the subsequently transplanted tumor grows with untrammeled or natural vigor, unimpeded by its contaminating virus.[21,25] This possibility was considered with the Brown-Pearce rabbit tumor XYZ material. A Lilac rabbit (resistant strain), in which a Brown-Pearce transplant had grown subcutaneously, regressed and disappeared, was sacrificed. Blood serum from this "immune" rabbit was mixed in equal parts with XYZ material and allowed to stand for some hours in a kitchen type ice box. This immune serum-XYZ mixture injected prior to tumor transplantation enhanced the XYZ effect. If an inert contaminating virus were present, then, the virus-serum mixture perhaps should have lessened the XYZ effect instead of increasing it. Also probably every strain of Brown-Pearce tumor throughout the world today was derived from animals which had had XYZ material before tumor inoculation and shipped (by AEC) to various places. Our own strains came from several generations of such XYZ animals yet the XYZ material may be obtained with regularity and ease from the frozen tissue. If a virus, it is inert in the sense that no fatalities or clinical disease follows its injection, and dynamic in the sense that metastases and local growth of the homologous tumor are enhanced by its injection. The recent experimental work of Kaplan and Murphy on insufficient radiation inducing more metastases is very suggestive of the XYZ effect.[19] The Brown-Pearce material is almost equally as effective when given 2 weeks after tumor transplantation as 2 weeks before.[1] The XYZ effect does not result when Berkefeld or Seitz filtrates of fresh tumor[22,23] or nuclei and cytoplasm of fresh tumor[24] are injected prior to tumor transplantation. A single injection of the XYZ factor into immune rabbits which had been tested and retested by inoculations of Brown-Pearce tumor broke down the resistance of 33% of the animals upon subsequent challenge with fresh tumor tissue.[1]

Summary and conclusions. 1. Twelve experiments were made with mouse mammary carcinoma Eo771 (C57 black origin) using 250 C57 black mice. Complementary use was made of mouse mammary carcinomata 241-5, 241-16 (C57 black origin), 15091a (Line A origin), and Barrett (C3H origin).

2. A highly selective XYZ factor (entirely comparable with those described for the Brown-Pearce rabbit tumor and Bashford mouse mammary carcinoma 63), resulting in enhanced tumor growth, was found to be uniformly present in the frozen Eo771 tumor tissue. The XYZ effect was present when the XYZ factor was derived from the homologous tumor and absent when derived from a heterologous tumor, even from other mammary carcinomata of the same highly inbred C57 black strain. This high tissue selectivity of the XYZ factor was not limited or determined by the host into which the tumor cells were transplanted. The factor was equally effective in homologous or heterologous transplant-

20 Taylor, M. J., and MacDowell, E. C., *Cancer Research*, 1949, **9**, 144.

21 Kritzler, Robert A., Mulliken, B., and Turner, Joseph C., *Cancer Research*, 1949, **9**, 74.

22 Casey, Albert E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 111.

23 Drysdale, George R., and Casey, Albert E., unpublished experiments.

24 Skipper, Howard, Edwards, P. C., Drysdale, George R., and Casey, Albert E., unpublished experiments.

25 Bang, F. B., and Gey, G. O., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 78.

ed hosts, so long as the tumor cells were viable in the foreign host.

3. XYZ factors have thus far been proven for only 4 transplanted neoplasms of mice and rabbits all of which are Grade III-IV by histologic grading. No such factors have been observed in adult tissues, leukemias, benign and low grade tumors.

Received July 27, 1949. P.S.E.B.M., 1949, **72.**

In vitro Determination of Bacterial Sensitivity to Aureomycin. (17340)

PAUL M. BEIGELMAN (Introduced by L. A. Rantz.)

From Department of Medicine, Stanford University School of Medicine, San Francisco. Calif.

Results of aureomycin sensitivity studies of various microorganisms have deviated greatly from one another as reported by different laboratories. This has been best exemplified by wide divergences in the reported sensitivities of staphylococci to this agent. Paine and associates[1] employing both tube dilution and plate methods, noted that 22 strains of *S. aureus* required 1 to 2 μg of aureomycin per ml for inhibition. The 5 remaining strains were inhibited by higher concentrations of the antibiotic, ranging up to 12.5 μg per ml. The type of media used was not described. Price and collaborators[2] reported *S. aureus* to be inhibited by a range of .09 to 25 μg of aureomycin per ml. The determinations were made following incubation of the organisms in penicillin assay broth for 24 hours. Bryer and associates[3] stated that 0.6 μg of aureomycin per ml inhibited staphylococci. Their report contained no statement about the method of determination. Twenty-one strains of this group of organisms tested by Lankford and Lacy[4] were inhibited by .05 to 0.2 μg of aureomycin per ml. Bacto-tryptose agar was the medium employed. With a tryptose broth turbidimetric method and 50% inhibition of growth in 18 hours as the end-point, the most sensitive strain was inhibited by .012 μg of aureomycin per ml and the most resistant by .033 μg per ml.

This study is an investigation of possible causes for discrepancies in the results obtained by others. In addition, a search has been made for a simple and reliable method of determining bacterial sensitivity to aureomycin.

Methods. Growth curves of *S. aureus,* enterococcus, *S. panama,* and *E. coli* were determined by a turbidimetric method. 0.1 ml inocula of 18 hour broth cultures were added to 18 mm test tubes containing 10 ml of tryptose broth (Difco). Aureomycin crystals were dissolved in cold sterile distilled water, diluted in tryptose broth, and added to the system immediately.* The final concentrations were .01, .02, .05, 0.1, 0.2, 0.5, 1, 2, 5, and 10 μg per ml. A control tube without aureomycin was included in each determination. The cultures were incubated at 37°C and the turbidity was measured in a Coleman Junior spectophotometer with a 650 m filter. Values of less than 0.1 unit of optical density indicated marked bacteriostasis and were chosen as end-points.

Tryptone glucose extract agar with para-aminobenzoic acid (Difco) was the medium used for the plate method. Aureomycin was incorporated in this medium in final concentrations identical with those described above plus 20 and 50 μg per ml, and 18 hour broth cultures were streaked on the plates. Readings were made at various intervals of time,

[1] Paine, T. F., Collins, H. S., and Finland, M., *J. Bact.,* 1948, **56**, 489.

[2] Price, C. W., Randall, W. A., and Welch, H., *Ann. New York Acad. Sci.,* 1948, **51**, 211.

[3] Bryer, M. S., Schoenbach, E. B., Chandler, C. A., Bliss, E. A., and Long, P. H., *J.A.M.A.,* 1948, **138**, 117.

[4] Lankford, C. E., and Lacy, H., *Texas Rep. Biol. Med.,* 1949, **7**, 111.

* Solutions of aureomycin in water retained full potency if stored in the frozen state.