

conclusion that resistance to penicillin in Group A and C streptococci is not likely to develop, it would be of great clinical importance. However, it should be emphasized that these observations on the group and strain behavior of artificially resistant streptococci, while interesting and valid, must not be assumed to apply by analogy to natural resistance.

Summary. 1. Five strains of Group C streptococci were subcultured serially on medium containing penicillin in an effort to induce resistance. Three of the 5 strains developed a 14- to 16-fold increase after 60 transfers. Two strains demonstrated only a 2- to 4-fold increase after similar subcultures.

2. No increased resistance was induced by serial transfers on control medium.

3. Acquired resistant organisms maintained

their resistance on serial subcultures on blood agar medium or on serial passages through mice.

4. Decreased mouse virulence was shown by all resistant strains of streptococci. Relatively little change appeared in the control organisms. Lost virulence was partially restored in 2 strains by passage in normal mice.

5. The group specific precipitinogen was demonstrable in only 2 of the 5 resistant strains, but was present in 4 control organisms.

6. Transient changes in the colonial appearance, and changes in hemolysis from beta to alpha or gamma were demonstrated by all strains when grown on maximal concentrations of penicillin. These colonies reverted to the parent type when subcultured on blood agar.

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Propagation of the Mammary Tumor Milk Agent in Tumors from C₅₇ Black Mice.*

JOHN J. BITTNER.

From the Division of Cancer Biology, Department of Physiology, University of Minnesota Medical School, Minneapolis, Minn.

If females of the C₅₇ black strain obtained the mammary tumor milk agent by nursing females of cancerous strains, mice of some sublimes may, when they are maintained as breeders, show incidences of mammary cancer in excess of 50%¹⁻⁴ although mice of this strain are generally regarded to have a "low susceptibility" for spontaneous mammary

cancer. In 2 studies it was determined that the incidence may be approximately as high,⁴ or even higher,² in the progeny than in the mice of the fostered generation, demonstrating that the females may propagate and transmit the milk agent. Fostered females and their descendants of a line of the C₅₇ black strain which gave a low incidence of spontaneous tumors have also been found to transmit the milk agent.⁵

The milk agent, one of the 3 causative factors for mammary cancer in mice,⁶ may be

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¹ Bagg, H. J., *International Cancer Research Foundation, Report of Activities During 1939*, p. 14.

² Fekete, E., and Little, C. C., *Cancer Research*, 1942, **2**, 525.

³ Andervont, H. B., *J. Nat. Cancer Inst.*, 1943, **3**, 359.

⁴ Haagensen, C. D., and Randall, H. T., *Cancer Research*, 1945, **5**, 352.

⁵ Bittner, J. J., *J. Nat. Cancer Inst.*, 1940, **1**, 155.

⁶ Bittner, J. J., *Public Health Rep.*, 1939, **54**, 1590.

TABLE I.
Activity of the Milk Agent in Spontaneous Mammary Tumors from C57 Blk. Mice, Line 6.

Tumor No.	Date injected	Gm.Eq. inj.	No.	% Ca	% living
7476 (A ₂)*	6/27/46	1/50	19	79	16
		1/1000	13	46	21
7477 (A ₂)*	"	1/50	29	55	14
		1/1000	25	60	36
7478 (Z ₁)	"	1/100	31	58	23
7788 (Z ₁)	3/26/47	1/50	56	30	67
		1/1000	32	25	69

* Tumors inoculated.

TABLE II.
Comparative Activity of the Milk Agent in Tumors from C57 Blk. Mice.

Tissue extracted	Gm.Eq.inj.	Tumor No. 7476			Tumor No. 7477		
		No.	% Ca	% liv.	No.	% Ca	% liv.
Spontaneous tumors	1/50	19	79	16	29	55	14
	1/1000	13	46	21	25	60	36
Transplants: after 1 passage	1/50	9	67	22	29*	100	—
	1/1000	21	48	29	20	65	25
Transplants: after 10 passages	1/10	38	39	58	38	66	34
	1/1000	44	25	73	44	16	82

* Average cancer age, 371 days.

recovered from extracts of spontaneous mammary cancer⁷⁻⁹ from mice of cancerous strains and has been found to survive for at least 10 passages of these tumors in mice which did not themselves have the milk agent.¹⁰

In this study we have investigated the presence of the milk agent in 4 spontaneous mammary tumors which developed in mice of Line 6 of the C₅₇ black stock. Two tumors, Nos. 7476 and 7477, arose in the progeny of a female which had obtained the milk agent from a foster mother of the cancerous A strain. The mother of the C₅₇ black mice died cancerous. The other tumors, Nos. 7478 and 7788, developed in females which had been nursed by a female of the cancerous C₃H stock. In addition, tumors 7476 and 7477 were transplanted into mice of the C₅₇ black

stock which had not received the milk agent, either from their mother or a foster mother. The transplanted tumors were tested after the first passage and also after the tenth passage for the survival of the milk agent.

The test animals were ZBC mice derived by reciprocal matings between mice of the fostered A and C₃H strains. Controls have been found to have few spontaneous mammary tumors whereas when the ZBC mice obtain the milk agent, high incidences have been observed.¹¹

The tumor extracts were all prepared in the same manner. The tissue was ground in a mortar with sand and suspended in either physiological saline or triple distilled water (1:10 by weight). The suspension was centrifuged for 7 to 10 minutes at approximately 2,500 r.p.m. and 1 cc of the supernatant, at various dilutions, was injected intraperitoneally into the test animals. The injected mice were continued as breeders to insure an adequate hormonal stimulation for the genesis

⁷ Bittner, J. J., *Science*, 1941, **93**, 527.

⁸ Barnum, C. P., Ball, Z. B., Bittner, J. J., and Vischer, M. B., *Science*, 1944, **100**, 575.

⁹ Andervont, H. B., and Bryan, W. R., *J. Nat. Cancer Inst.*, 1944, **5**, 143.

¹⁰ Bittner, J. J., Evans, C. A., and Green, R. G., *Science*, 1945, **101**, 95.

¹¹ Bittner, J. J., *AAAS, Research Conference on Cancer*, 1945, p. 63.

of mammary cancer.

The data are presented in Tables I and II.

The results demonstrate that the mammary tumor milk agent may be recovered from spontaneous mammary cancers which developed in mice of the C₅₇ black stock and may be propagated in the transplants of these tumors. Two of the tumors arose in mice which had received the agent from their mothers, again demonstrating that females of

the C₅₇ black strain, at least some sublines, may transmit the agent in the milk.

Summary. Mice of some sublines of the C₅₇ black stock may propagate and transmit the mammary tumor agent. The agent may be recovered from spontaneous mammary cancers which developed in mice of the strain and may survive for at least 10 passages of these tumors in mice without the agent.

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Effect of Bacterial Endotoxins on Carbohydrate Metabolism of Rabbits.*

ERNEST KUN AND C. PHILLIP MILLER.†

From the Departments of Pharmacology and Medicine, University of Chicago.

The injection into animals of heat-killed cultures of certain bacterial cells has been shown to produce marked hyperglycemia.^{1,2} Diphtheria toxin which has been studied more extensively³ causes a similar rise in blood sugar and depletion of tissue carbohydrate. Some investigators^{4,5} have concluded that diphtheria toxin affects metabolism by damaging the liver, causing increased glycolysis and diminished phosphorolysis. The biochemical mechanism by which the metabolites or cellular constituents of bacteria interfere with the metabolism of the infected animal is not well understood.

The experiments described below concern the effects of meningococcal and *Salmonella* endotoxins on the intermediate carbohydrate

metabolism of rabbits. As in experiments already reported,⁶ the experimental conditions may be defined as acute endotoxin poisoning. The effect of a lethal dose of endotoxin was studied during the short period of intoxication which terminated in the death of the animal in 1.5-3 hours. It is believed that, under such circumstances, the effect of the toxin is not significantly altered by spontaneous changes in metabolism.

Materials and Methods. The endotoxins were sterile, unpurified preparations made from washed bacterial cells by the method already described.⁶ The intravenous dose which was lethal in 2 to 3 hours was 20-50 mg per kg. Rabbits weighing 1.5-3 kg were used. The experimental and control animals were fasted for 24 hours before each experiment. No anesthesia was used. Blood samples were taken by cardiac puncture. Tissue analyses were carried out immediately after the death of the animal. Pairs of rabbits having almost identical weights were kept under the same environmental conditions throughout each experiment. One rabbit in each pair was injected with endotoxin and the other kept as a control and sacrificed when death occurred in the former. In some

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⁵ Taubenhaus, M., and Soskin, S., *J. Clin. Endocrin.*, 1942, **2**, 171.

⁶ Kun, E., *Proc. Soc. Exp. Biol. and Med.*, 1947, **66**, 197.