growth-promoting effect of low concentrations of the same complex. While the mechanism whereby an excess of thiamine or pantothenate may fail to improve work output is problematical, the diminished effect of high concentrations is apparently real.

Summary. The work output of the gastrocnemius muscle of the perfused frog has been determined at different levels of thiamine concentration in the perfusion fluid. The effects on the work output of adding thiamine pyrophosphate (co-carboxylase) and thiamine hydrochloride to the perfusion fluid has been compared. 1. The addition of thiamine hydrochloride to the perfusion fluid improved the work output of frog muscles. The degree of improvement increased with increasing concentrations of hydrochloride from 0.0001 to 0.001 mM per liter. 2. No further improvement in work output resulted when thiamine hydrochloride concentration was increased to 0.1 mM per liter. 3. Increasing the thiamine concentration of the perfusion fluid from 0.1 to 1.5 mM per liter progressively reduced the improvement in work output. 4. Thiamine pyrophosphate was also effective in increasing the work output in perfused frog muscles. 5. Thiamine pyrophosphate increased the work output at lower concentrations than did thiamine hydrochloride. 6. At concentrations of 0.001 mM per liter or above, the extent of the improvement in work output was no greater with thiamine pyrophosphate than with thiamine hydrochloride.

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X. Carcinoma of Mammary Gland Following Injection of Methylcholanthrene into Mice of NHO Strain.

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Adenocarcinomas of the mammary gland in mice have been known since 1854.[1] The usual degree of morphologic variation exhibited by mouse mammary gland tumors has been interpreted by Apolant,[2] and others,[3] as representing a series of sequential phases of a single pathological process. A continuous gradient may be observed from the benign adenomas to the adenocarcinomas and the undifferentiated medullary carcinomas. In general these tumors are only histologically malignant, with remote metastases and extensive invasion of adjacent tissues occurring only infrequently.

Numerous agents have been incriminated by the many investigators of the problem as having some influence on the origin of these tumors in mice. However, 5 influences have received rather widespread recognition. These influences may be briefly classified as (1) genetic; (2) hormonal; (3) the "milk" influence; (4) dietary factors; and (5) somatic mutation.

Recently mammary gland tumors which had been encountered only in genetically susceptible mice, either spontaneously or following hormone injection, have been duplicated following the subcutaneous injection of methylcholanthrene in sesame oil into female mice of certain strains which lack the milk influence, are spontaneously resistant to mammary gland tumors, and do not develop such tumors after estrogen treatment.[4,5,6] This observa-

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1 Crisp, *Tr. Path. Soc. London*, 1854, **5**, 348.

2 Apolant, H., *Arb. A. d. Königlden Inst. f. Exp. Ther.*, Frankfort, A. M., 1906, **1**, 7.

3 Dunn, T. B., 1945, Morphology and Histogenesis of Mammary Tumors, in Moulton, F. K., editor, *A Symposium on Mammary Tumors in Mice*, p. 13.

4 Strong, L. C., and Smith, G. M., *Yale J. Biol. and Med.*, 1939, **11**, 589.

5 Strong, L. C., and Williams, W. L., *Cancer Res.*, 1941, **1**, 886.

tion has been verified in several laboratories with various strains of resistant mice.[7,8,9,10,11]

In this investigation, mice of the NH and NHO strains have been used since they are known to be genetically resistant to mammary gland tumors. There has never been obtained a single case of mammary tumor in the control NH mice (under both breeding and non-breeding regimes), nor in the untreated descendants of methylcholanthrene-treated animals. Mice of the NH and NHO strains are, therefore, up to the present time, highly resistant to spontaneous breast tumors. This conclusion is based upon the following unpublished data: 476 virgin female mice of the original control NH stock autopsied between 150 and 820 days; 190 female mice used as breeders of the same control NH stock autopsied between 180 and 730 days; and 431 untreated female descendants of methylcholanthrene-treated mice autopsied between 150 and 730 days (375 non-breeders and 56 mice that had been used for breeders). In addition to these observations, it has been determined that, in an outcross of the original control NH stock mice to mice of the C_3H strain, spontaneous tumors of the mammary gland occur only in the F_1 female mice provided the mother came from the cancer-susceptible C_3H strain (maternal inheritance). It has been found out too (unpublished data) that when C_3H female mice are fostered onto NH mothers, they do not develop spontaneous tumors of the mammary gland.

Originally mammary gland tumors occurred sporadically following the injection of methylcholanthrene. At present, however, mammary tumors can be induced in at least 60% of the female mice of certain strains treated with the carcinogen. This was the incidence obtained in two sublines of the NHO strain[12] which had been genetically selected toward suppression of local methylcholanthrene-induced tumors such as fibrosarcoma, epidermoid carcinoma and rhabdomyosarcoma.[13] In the most recent observations on mice of two sublines of this selected strain, which were in the F_{14} to F_{18} generations following an outcross, 75 female mice out of a total of 125 injected subcutaneously with methylcholanthrene developed mammary gland tumors, either locally at the site of injection or in any other of the mammary gland regions.

These tumors induced by methylcholanthrene duplicate in every histologic detail the mammary tumors which occur in mice either spontaneously or after the injection of an estrogenic hormone. In addition to the usual histology, however, many of the tumors possess certain new characteristics such as metastasis to bone and invasion of adjacent tissues. Anaplasia and squamous metaplasia are observed with greater frequency than in tumors of the mouse mammary gland obtained by earlier means.

Upon examination of a typical example among the more undifferentiated tumors, it may be noted that the morphology varies considerably in different portions of a single tumor. In some areas the stroma is scanty, in others abundant. The tumor cells are hyperchromatic and may either be packed densely in solid sheets or occur singly or in small clumps in the abundant stroma. All traces of an acinar origin have been lost. Frequently, elongated cords of nearly spindle-shaped tumor cells, sometimes a single cell layer thick, may be observed. The tumor invades early the surrounding tissue and in some areas there seems to be no capsule. The tumor cells that invade the surrounding musculature and the neighboring bony structures are very anaplastic. The metastatic nodules in bone erode the cortex and fill the medullary cavity, replacing the bone marrow. The mammary gland in the neighborhood of one of these ana-

[6] Kirschbaum, A., and Strong, L. C., *Cancer Res.*, 1942, **2**, 841.

[7] Orr, J. W., *J. Path. and Bact.*, 1943, **55**, 483.

[8] Bonser, G. M., 1939, *Sixteenth Ann. Rep. Brit. Empire Cancer Campaign*, p. 165.

[9] Bonser, G. M., and Orr, J. W., *J. Path. and Bact.*, 1938, **49**, 171.

[10] Bonser, G. M., and Orr, J. W., 1940, *Seventeenth Ann. Rep. British Empire Cancer Campaign*, p. 123.

[11] Kirschbaum, A., and Bittner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 18.

[12] Strong, L. C., *Am. J. Cancer*, 1940, **39**, 347.

[13] Strong, L. C., *Yale J. Biol. and Med.*, 1944, **17**, 289.

TABLE I.
Includes the Autopsy Records on Female Mice of the NH and NHO Strains in Reference to Mammary Gland Tumors. Mcl. means 1 mg of methylcholanthrene dissolved in 0.1 ccm sesame oil and injected subcutaneously at 60 days.

Strain	Class	Generations inbreeding	Type female	No. mice	Mammary tumors	Autopsy age range
NH	Control	F_{10}-F_{20}	Breeder	198	0	180-730
		F_{10}-F_{20}	Virgin	476	0	150-820
NHO	Untreated descendants	F_{10}-F_{18}	Breeder	56	0	150-730
		F_{10}-F_{18}	Virgin	375	0	150-730
NHO	Injected	F_{14}-F_{18}	Breeders	125	75	84-525
Total controls				1105	0	
Exp. (Mcl. inj.)				125	75	

plastic tumors appears to be quite hyperplastic and undergoing squamous metaplasia.

Several conclusions may be drawn from the results of this investigation to date:

1. The series of breast tumors occurring under spontaneous conditions in genetically susceptible female mice and following the injection of estrogenic hormones in male mice can be duplicated in apparently resistant female mice of certain strains following the subcutaneous injection of methylcholanthrene.

2. The injection of methylcholanthrene can therefore replace spontaneous genetic susceptibility. These mice may be genetically susceptible to this particular agent (methylcholanthrene) even though resistant to others but the manner of inheritance has not, as yet, been ascertained. The suggestion should be entertained that the present experiment may represent evidence of agent-specificity of genetic control.

3. The tumors arising in female mice genetically resistant to spontaneous mammary tumors following the injection of methylcholanthrene possess 4 characteristics not present or less pronounced in the tumors of genetically susceptible female mice: (a) they show an increased tendency to undergo squamous metaplasia; (b) many of them (approximately 18%) show rather pronounced anaplastic changes; (c) these anaplastic tumors invade surrounding normal tissues rather extensively and (d) do metastasize to bone.

4. These malignant breast tumors in mice appear closely to simulate some of the characteristics of human mammary carcinomas.

Much further work is necessary on this phenomenon of the production of anaplastic carcinoma, including an investigation of the roles of the various agents, such as the milk influence and estrogens, which are known to influence the other types of tumors of the mammary gland in mice. It is hoped to demonstrate whether the action of methylcholanthrene actually involves a reversal of genetic resistance or merely permits the manifestation of a new type of agent-specific susceptibility which will not spontaneously yield mammary tumors.

Summary. 75 female mice of a total of 125 injected subcutaneously with 1 mg of methylcholanthrene at 60 days of age, developed carcinomas of the mammary gland. The mice belonged to the F_{14}-F_{18} generations of inbreeding. This high incidence of mammary gland tumors was made possible by the suppression of local tumors through the process of genetic selection toward resistance to such local tumors. These induced mammary gland tumors duplicate the series of tumors produced spontaneously in genetic susceptible strains of mice such as the A and the C_3H. In addition, however, the tumors arising in genetically resistant female mice of this experiment following the injection of methylcholanthrene possess 4 characteristics not present or less pronounced in the tumors of genetically susceptible female mice: (a) they show an increased tendency to undergo squamous metaplasia; (b) many of them (approximately 18%) undergo rather pronounced anaplastic changes, (c) these anaplastic tumors

invade surrounding normal tissues rather extensively and (d) do metastasize to bone. These new malignant breast tumors in mice appear closely to simulate some of the characteristics of human mammary carcinomas.

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Effect of p-Aminobenzoic Acid on Growth of Rickettsiae and Elementary Bodies, with Observations on Mode of Action

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In a previous report,[1] publication of which was delayed for reasons of military security, it was shown that the growth of typhus rickettsiae in the yolk sac of the developing chick was markedly inhibited by p-aminobenzoic acid.* We demonstrated further that the best inhibition of rickettsial growth was obtained only when maximal dosages of paba were used, which in the case of the chick embryo was 2-4 mg per egg. The lowest amount of paba which would give a certain inhibition was 0.2-0.4 mg per egg. These experiments were an outgrowth of a preliminary report by Snyder, Maier, and Anderson[2] in which data (on mice infected with murine typhus) were presented which suggested that paba might be of therapeutic value. On the basis of the fact that maximal dosages of paba are necessary for the inhibition of rickettsiae, large amounts of paba were then administered to humans infected with typhus, and good therapeutic results were obtained (Yeomans *et al.*)[3] Greiff, Pinkerton, and Moragues[4] have studied the effect of enzyme inhibitors and activators (penicillin, para-aminobenzoic acid, sodium fluoride, and vitamins) on the multiplication of typhus rickettsiae, and have also found that paba inhibits rickettsial growth.

In our first report it was mentioned that a similar inhibition of rickettsial growth was obtained when eggs that had been infected with Rocky Mountain spotted fever were treated with paba. A more detailed analysis of the action of this drug on different members of the spotted fever group showed not only that the inhibition was more striking in many cases than was obtained with typhus, but that differences in magnitude of the response of different kinds of rickettsiae to paba could be correlated with their antigenic relationships. It is the purpose of the present report to present these quantitative aspects of the growth of different kinds of rickettsiae (and some elementary bodies) in the presence of paba. Data are also included which have a bearing on the possible mode of action of paba.

Methods. The methods used in measuring the effect of paba on the growth of rickettsiae were similar to those previously described.[1] Hen's eggs which had been incubated 8 days and contained normal-appearing, vigorous embryos were selected and divided into two lots. Each egg of the first, or control, lot received an injection of 0.5 cc of sterile distilled water in the yolk sac, and each egg of the test series received a similar injection of 0.5 cc of a sterile solution of paba in distilled water. Immediately after the injection with these solutions, each egg was infected by injecting

[1] Hamilton, H. L., Plotz, H., and Smadel, J. E., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 255. (Report to the Director of the U. S. A. Typhus Commission, 16 December, 1943.)

* Hereafter referred to as paba.

[2] Snyder, J. C., Maier, J., and Anderson, C. R., Report to the Division of Medical Sciences, National Research Council, Washington, D.C., 26 December, 1942.

[3] Yeomans, A., Snyder, J. C., Murray, E. S., Zarafonetis, C. J. D., and Ecke, R. S., *J. A. M. A.*, 1944, **126**, 349.

[4] Greiff, D., Pinkerton, H., and Moragues, V., *J. Exp. Med.*, 1944, **80**, 561.