

- b. 1 cc of 24-hr broth culture of *E. coli*.
 - c. 1 cc of 10% suspension of vaccinia virus.
 - 3. Virulence-enhancing agent.
 - a. 2 cc of 5% gastric mucin.
- Group 6. Control group. Dermal and testicular tumors of active myxoma virus and active fibroma virus injected into normal rabbits.

Results. The Berry transformation was successful only in Group 3 of Experiment I. Rabbit 1 exhibited generalized myxomatosis on the 12th day after injection and subsequently recovered. Testicular tissue removed from this rabbit on the 7th day following injection produced typical fatal myxomatosis in two normal rabbits. The possibility of accidental infection of this transformation rabbit was eliminated because there were no myxomatous rabbits in the laboratory at the time.

Typical fibromatosis and myxomatosis were produced in the control group.

Attempts to facilitate the transformation by the use of attenuated fibroma virus and small doses of active fibroma virus were not successful.

Heat-inactivated myxoma virus was not reactivated by inflammatory, reducing, and virulence-enhancing agents, or by serial passage.

Discussion. The occurrence of the Berry transformation in only one of 21 rabbits emphasizes the observations of other workers that this transformation is not accomplished easily.^{1,2,3,5,6} Our inability to reactivate heat-inactivated myxoma virus with diverse agents amplifies the evidence of Berry^{7,8} and Gardner and Hyde.⁶ Additional data are required to prove whether fibroma virus reactivates heat-inactivated myxoma virus or is actually transformed into active myxoma virus.

⁷ Personal communication.

⁸ Berry, G. P., and Dedrick, H. M., *J. Bact.*, 1936, **32**, 356.

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Initiation of Secretory Changes in Transplanted Mammary Adenocarcinoma of the Rat by Pituitary Lactogenic Hormone.*

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Secretory phenomena in a transplanted mammary adenocarcinoma (R2426), maintained in inbred August rats, have been described following treatment of hosts of either sex with 0.166 mg estradiol benzoate or 0.2 mg estradiol dipropionate twice weekly.¹ Subsequent experiments have demonstrated that daily injections of considerably smaller quantities of the hormones are equally effective in initiating these changes, which

varied in degree from isolated foci of secretory vacuoles in the neoplastic cells, evident only on microscopic examination, to extensive transformation of tumors into lactating cystic masses containing thick milk-like fluid. Neoplasms of the latter type bore little resemblance to the original adenocarcinoma. Tumors were not influenced by the normal hormonal variation incident upon age or sex,² nor did castration, pregnancy and lactation, or testosterone propionate affect either their growth or their histologic characteristics.¹

Experimental. It appeared of interest to ascertain the influence of the lactogenic

* Anterior pituitary lactogenic hormone was generously furnished by the Research Department of Parke, Davis & Co., Detroit, Mich.; progesterone and estradiol dipropionate by Ciba Pharmaceutical Products, Inc., Summit, N.J.

¹ Eisen, M. J., *Cancer Research*, 1941, **1**, 457.

² Eisen, M. J., *Am. J. Cancer*, 1940, **39**, 36.

TABLE I.
Effect of Anterior Pituitary Lactogenic Hormone on Transplanted Mammary Adenocarcinoma (R 2426) in Nursing Rats.

Rat	Age of animals when tumor transplanted, days	Littered, days after transplantation	No. nursed	No. days nursing when treated	Treatment	Killed, days after transplantation	Result
1	80	40	5	7	4 x 40 units	52	Secretory foci
2	80	47	8	12	6 x 40 "	66	" "
3	185	28	4	3	6 x 80 "	38	" "
4	213	15	3	10	5 x 80 "	31	No change

TABLE II.
Effect of Anterior Pituitary Lactogenic Hormone, Estradiol and Progesterone on Transplanted Mammary Adenocarcinoma (R 2426).

No. of rats	F M	Status	Previous treatment	Lactogenic hormone	Result
2	2	Intact	None	4 x 40 units	No secretion
2	2	"	Progesterone	"	" "
6	6	Castrated	"	"	" "
2	2	Intact	Estradiol, Progesterone	"	Secretory foci
3	5	"	"	None	" "

factor of the pituitary gland on a mammary tumor of this type which reacts so strikingly to the influence of exogenous estrogen. The biologic characteristics of this lactogenic hormone have been reviewed in detail by Riddle.³ When employed clinically in cases of deficient lactation the results have proved controversial, some investigators^{4,5} having recorded a favorable influence, while others⁶ have denied any specific action.

The possibility that it might have some action on this carcinoma was tested in 36 tumor animals: 4 suckling females (Table I) and 32 other rats, including non-suckling females, normal males, both normal and castrated females and males following previous treatment with progesterone, and intact animals of either sex treated earlier with estradiol and progesterone (Table II). The effects of estradiol and progesterone alone were also examined.

The suckling rats bore subcutaneous tumors in the right flank and had successfully cared for their litters for 3 to 12 days. Suckling was permitted to continue during the period of treatment. From one-half to one cc (40 to 80 crop gland units) was injected subcutaneously at a distance from the tumors daily for from 4 to 6 days and the animals were sacrificed 48 hr after the last treatment. Evidence of secretory activity was found in the tumors of 3 among 4 of these rats. The single unaffected tumor had been transplanted after the onset of pregnancy. Whether the sensitivity of the others was related to the longer and more complete normal hormonal stimulation to which they had been subjected from the beginning of pregnancy cannot be stated definitely on the basis of the available data.

The alterations could not be perceived with certainty upon gross examination, but under the microscope they were demonstrable in many areas throughout the tumors. In the altered zones some or all the cells of a considerable number of alveoli contained distinct secretory vacuoles in the area bordering the acinar cavities. These functioning neo-

³ Riddle, O., *J. Am. Med. Assn.*, 1940, **115**, 2276.

⁴ Kurzrok, R., Bates, R. W., Riddle, O., and Miller, E. G., Jr., *Endocrinology*, 1934, **18**, 18.

⁵ Ross, J. R., *Endocrinology*, 1938, **22**, 429.

⁶ Stewart, H. L., Jr., and Pratt, J. P., *Endocrinology*, 1939, **25**, 347.

plastic acini were more regular and rounded in shape than those in the unchanged tumor zones.

The treatment exerted no measurable effect upon the amount of milk secreted by the mamma, nor did histologic examination disclose any departure from the manifestations normally occurring in lactating mammary tissue.

As for the non-suckling and the male animals, pituitary lactogenic hormone in 4 daily doses of 40 units each, administered to 2 females and 2 males bearing transplanted tumors of 42 days, produced no changes in the neoplasms. Likewise similar treatment of 6 spayed females and an equal number of castrated males following 15 injections of 0.25 mg each of progesterone dissolved in sesame oil, or of 2 intact females and 2 males that had received 17 injections of progesterone, exerted no influence on the transplanted cancers. The progesterone, given 3 times weekly, was begun 10 days after transplantation, the lactogenic factor 3 days following the final treatment with the former. Tumors of animals that received only progesterone failed to show changes.

While the administration to 3 normal females and 5 males of 17 injections, given 3

times weekly, of 0.25 mg of progesterone and 0.025 mg of estradiol dipropionate resulted in extensive secretion in the tumors, the alterations did not differ from those induced by this quantity of estradiol alone. Treatment of 2 normal females and an equal number of males with 4 daily doses of 40 units each of pituitary lactogenic hormone, begun 3 days following the completion of the previously described course of progesterone and estradiol, did not enhance the effects of the latter combination of hormones.

Summary. The daily injection of 40 to 80 crop gland units of pituitary lactogenic hormone for 4 to 6 days into rats suckling their young induced secretory changes in a transplanted mammary adenocarcinoma. The hormone produced no effect on the tumors of non-nursing females or normal males. Progesterone also exerted no influence, nor did it affect, when given simultaneously with estradiol dipropionate, the secretory phenomena induced in the neoplastic cells by the latter substance alone. Pituitary lactogenic hormone failed to induce secretion in the tumor cells of animals previously treated with progesterone, and did not enhance the alterations produced by the combined administration of progesterone and estradiol.

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Absorption and Excretion of Sulfamethyldiazine (2-Sulfanilamido-4-methyl-pyrimidine) in Human Subjects.

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Sulfamethyldiazine (2-sulfanilamido-4-methyl-pyrimidine) was prepared and characterized by Roblin *et al.*¹ at the same time that they reported on sulfadiazine. Both these drugs were found to be active in mice against experimental pneumococcal and streptococcal infections in mice. The former

was found to be $2\frac{1}{2}$ times as soluble as the latter in water at 37°C and its N4-acetyl derivative was also reported to be twice as soluble as that of sulfadiazine. Maximum blood levels in mice of sulfamethyldiazine were found to be slightly higher than those obtained on the same dose of sulfadiazine. The 4-6-dimethyl derivative² is considerably

¹ Roblin, R. O., Jr., Williams, J. H., Winnek, P. S., and English, J. P., *J. Am. Chem. Soc.*, 1940, **62**, 2002.

² Roblin, R. O., Jr., Winnek, P. S., and English, J. P., *Ibid.*, 1942, **64**, 567.