mucopolysaccharide excretion by the uronic acid content of a total CTAB precipitate but did not purify this material. The purity and quantitative yield of the mucoprotein fractions obtained in this study are indicated by the equimolar ratios of hexuronic acid and hexosamine, by the nitrogen content and by the radioactivity. The fractions appear free of serum proteins, glycoproteins and other large molecules found in urine.

*Summary.* A method for isolation of sulfated acid mucopolysaccharides (AMPS) and corresponding mucoproteins from human urine by absorption on and precipitation with cetyltrimethylammonium bromide is described. After *in vivo* labelling with radiosulfur nearly all of the bound radioactivity was found in 2 fractions, one insoluble in 66% alcohol and the other soluble in 66% alcohol, but insoluble in 84% alcohol. The first is essentially free AMPS containing less than 10% protein while the second is a mucopolysaccharide-protein complex containing about 87% protein. Digestion of the second fraction with proteolytic enzymes yields a nondialyzable AMPS with about 26% protein. Chemical analysis indicates no other high molecular weight urinary substances are present in these preparations.

1. Mörner, K. A. H., *Skand. Arch. Physiol.*, 1895, v6, 332.

2. Hammerman, D., Hatch, F. T., Reife, A., Bartz, K. W., *J. Lab. Clin. Med.*, 1955, v46, 848.

3. King, J. S., Jr., Boyce, W. H., Little, J. M., Artom, C., *J. Clin. Invest.*, 1958, v37, 315.

4. Anderson, A. J., McClagan, N. F., *Biochem. J.*, 1955, v59, 638.

5. Tamm, I., Horsfall, F. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 108.

6. ———, *J. Exp. Med.*, 1952, v95, 71.

7. Di Ferrante, N., Rich, C., *J. Lab. Clin. Med.*, 1956, v48, 491.

8. Stidworthy, G. H., Ph. D. Thesis, 1961, Univ. of Okla.

9. Rigas, D. A., Heller, C. G., *J. Clin. Invest.*, 1951, v30, 853.

10. Berggärd, I., *Clin. Chem. Acta*, 1961, v6, 413.

11. Miettinen. T., *Scand. J. Clin. Lab. Invest.*, 1961, v13, Sup. 61.

12. Björnesjö, K. B., Werner, I., Odin, L., *ibid.*, 1959, v11, 238.

13. Dutta, S. K., Jones, A. S., Stacey, M., *Biochem. Biophys. Acta*, 1953, v10, 613.

14. Scott, J. E., in *Methods of Biochemical Analysis*, D. Glick, Editor, Interscience, New York - London, 1960, vVIII, p145.

15. Eastoe, J. E., in *Biochemist Handbook*, C. Long, Editor, Van Nostrand, Toronto, New York, London, 1961, p726.

Received December 26, 1962. P.S.E.B.M., 1963, v112.

## Relation Between Sensitivity to Lactogenic Hormones and Tumorigenesis in Hyperplastic Mammary Nodules in C3H/Crgl Mice.* (28191)

HOWARD A. BERN

*Department of Zoology and Cancer Research Genetics Laboratory, University of California, Berkeley*

The hyperplastic alveolar nodule is the recognizable preneoplastic lesion in the mouse mammary gland, and tumors arise with high frequency when such nodules are transplanted into mammary fat pads which have been cleared of their normal parenchyma(1). Nodules, in general, appear to be more responsive to hormones than comparable normal tissues (2); their very existence as lobuloalveolar structures in an inactive gland is based upon this increased sensitivity. However, there is variation in sensitivity among nodules *in situ* (3,4) and among outgrowths that develop when these nodules are transplanted(5). We have previously raised the question as to whether the neoplastic change is more likely to occur in hyperplastic alveolar nodules showing greater hormone sensitivity than in nodules showing lower hormone sensitivity, or *vice versa*(6). To examine this question, intact mice have been subjected to various

* Aided by USPHS grant and by a research professorship in Miller Institute for Basic Research in Science, 1961.

TABLE I. Comparison of Tumorigenesis from Mammary Nodules with Different Degrees of Lactogenic Response to Hormones.

| Donors | | | | Hosts | | |
|---|---|---|---|---|---|---|
| No. of mice | Hormone treatment | Daily dosage ($\mu$g) | Duration of treatment (days) | No. of mice | No. of nodules showing tumors/No. of nodules showing hyperactive outgrowth "Milk-secreting" | "Not milk-secreting" |
| 5 | Cortisol acetate | 50 | 3 | 12 | 4/9 | 3/9 |
| 2 | " " | 50 | 5 | 7 | 1/5 | 3/7 |
| 8 | " " | 75 | 3 | 26 | 8/21 | 8/20 |
| 1 | Corticosterone acetate | 500 | 3 | 4 | 0/4 | 1/3 |
| 4 | *Idem* | 1000 | 3 | 7 | 0/7 | 1/7 |
| 3 | " | 1500 | 3 | 9 | 2/9 | 1/9 |
| 5 | Deoxycorticosterone acetate | 1000 | 5 | 17 | 8/17 | 4/12 |
| 3 | *Idem* | 1500 | 3 | 10 | 2/7 | 1/10 |
| 7 | Estradiol-17$\beta$ | single 2.5 mg pellet | 10 | 15 | 5/14 | 3/10 |
| 5 | *Idem* | 1 | 10 | 20 | 7/18 | 2/14 |
| Total 43 | | | | 127 | 37/111=33% | 27/101=27% |

steroid treatments which are capable of inducing milk secretion in many nodules(3,4). Nodules with and without milk secretion were transplanted to determine any relation between lactogenic response and tumor-forming ability.

*Materials and methods.* Nodules selected from 43 variously treated non-pregnant, multiparous, tumor-bearing, female C3H/Crgl mice were transplanted into both left and right inguinal mammary fat pads of 127 3-week-old female mice; the fat pads were cleared of their mammary parenchyma(1) immediately before transplants were placed. Each animal received on one side a nodule which showed some milk secretion and on the other side a nodule showing little or no secretion. Thus, each host mouse comprised a single paired experiment. However, owing to the occasional occurrence of parenchymal regrowth as a result of incomplete removal of the mammary anlage, of ingrowth of parenchyma from the No. 5 gland, or of failure of a transplant to take on one side, the number of successful "internally paired" experimental individuals was reduced. Accordingly, comparison has been made of the number of tumors arising from more actively secretory nodules with that arising from less actively secreting ones in the several treatment groups.

The various steroid hormones and dosages used are summarized in Table I. The steroids were given subcutaneously, generally as 0.2 ml aqueous suspension (0.4 ml in the case of 1500 $\mu$g deoxycorticosterone; as a pellet with approximate calculated absorption of 5 $\mu$g daily in one estradiol-treated group).

The hosts were maintained as virgins and given no treatment during the experiment. Animals were killed 16-18 weeks after transplantation, or earlier if a palpable tumor appeared in the transplant area. Complete wholemount preparations of the mammary apparatus were made.

*Results and discussion.* Mammary fat pads that showed any of the following were eliminated from consideration: (1) incomplete removal of the original parenchyma ("regrowth"); (2) ingrowth of normal parenchyma from the adjacent posterior (No. 5) gland sufficient to interfere with outgrowth from transplant; (3) entirely normal-appearing outgrowths or no outgrowth from transplant. All successful transplants showed some hyperactive outgrowth(1). Several subcategories of growth patterns are included under hyperactive (hyperplastic) outgrowth: expansion of the original lobuloalveolar-appearing nodule explant; ductal proliferation showing major areas of hyperplastic growth; lobuloalveolar outgrowth resembling prelactational or lactational mammary gland (cf. 1).

Table I summarizes the data from the several groups. In general, it is apparent that the various lactogens used did not permit distinction of tumor-forming ability between those nodules showing more milk secretion and those showing less. The action of the

steroids used may be in part indirectly mediated through the hypophysis and certainly would involve synergism with endogenous hypophyseal hormones(7). Inasmuch as nodules are generally identified for experimental studies in our laboratory by nature of their secretion of milk in response to a lactogenic hormone mixture, it is important to note that use of this response does not mean that we are selecting nodules with greater tumor-forming ability.

It would be incorrect to conclude that there is no relation between hormone sensitivity and neoplastic potential, but only that this particular criterion—the response of nodules to steroids in terms of milk secretion—indicates no particular relation, direct or inverse. It is possible that the degree of responsiveness of the nodules *in situ* to hormones and their tumor-forming ability are two independently variable characteristics; this would be in accord with studies on outgrowths from transplanted nodules, where there is no indication of a relation between degree of hormone sensitivity and frequency of tumor formation(8). Other properties of nodule outgrowths, including histologic architecture, secretory activity, growth rate, and chromosome number(5,9) also appear to be variables which may have no direct relation to neoplastic potential. Accordingly, the same may hold true for many properties of primary nodules *in situ*: morphology, mitotic index (Banerjee, unpubl.), dependence on insulin for glucose utilization (Moretti, unpubl.) and, as shown herein, degree of responsiveness to lactogenic steroids.

*Summary.* Transplantation of mammary hyperplastic alveolar nodules showing various degrees of milk secretion as a result of prior treatment with lactogenic steroids revealed no relationship between hormone sensitivity and tumor-forming ability.

The important assistance of Virgil Finster, Sherry Norris and Naomi Lidicker is gratefully acknowledged.

1. DeOme, K. B., Faulkin, L. J., Jr., Bern, H. A., Blair, P. B., *Cancer Res.*, 1959, v19, 515.

2. DeOme, K. B., Bern, H. A., Elias, J. J., Nandi, S., Faulkin, L. J., Jr., *Proc. II Intl. Symp. Mammary Cancer*, Perugia, 1958, p595.

3. Bern, H. A., Nandi, S., Finster, V., *Experientia*, 1959, v15, 155.

4. Nandi, S., Bern, H. A., DeOme, K. B., *Acta Unio Internat. c. Cancrum*, 1960, v16, 211.

5. Blair, P. B., DeOme, K. B., Nandi, S., In *Biological Interactions in Normal and Neoplastic Growth*, M. J. Brennan, W. L. Simpson, eds., Little, Brown, Boston, 1962, p371.

6. Bern, H. A., *Science*, 1960, v131, 1039.

7. Nandi, S., *J. Nat. Cancer Inst.*, 1958, v21, 1039; Bern, H. A., Nandi, S., *Prog. Exper. Tumor Res.*, v2, 90.

8. DeOme, K. B., Blair, P. B., Faulkin, L. J., Jr., *Acta Unio Internat. c. Cancrum*, 1961, v17, 973.

9. Banerjee, M. R., DeOme, K. B., *Cancer Res.*, in press.

Received December 26, 1962. P.S.E.B.M., 1963, v112.

## Methods for Reducing Duration of Parabiosis in Development of Tolerance to Skin Homografts in Mice. (28192)

ANATOLIO B. CRUZ, JR., LLOYD D. MACLEAN AND J. BRADLEY AUST
(Introduced by C. Martinez)
*Department of Surgery, Ancker Hospital, St. Paul and University of Minnesota Hospitals, Minneapolis*

Parabiosis has been shown to induce tolerance of homologous skin grafts between inbred mice(1,2,3,4). This apparently results from a continuous transfer of all transplantation antigens, presumably in the form of intact cells(2,3,4,5,7,8). It has been shown that the parabiotic state must be preserved for a critical length of time to obtain tolerance of homologous tissue(7,8). This period appears to vary among strains and it is also dependent on the degree of histocompatability difference between the parabionts(7). If the