Incorporation of P^{32} into Phosphatido-Peptide Fraction of Normal and Neoplastic Mouse Epidermis.* (26740)

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In 1959, Huggins and Cohn(1) reported studies on a phosphatido-peptide fraction from renal cortical tissue which was found to contain inositol, phosphorus, fatty acids, glycerol and amino acids presumably in peptide linkage. They also reported that this fraction exhibited a higher turnover rate in renal tissue when compared with phospholipid as measured by P^{32}. Distribution studies revealed that phosphatido-peptide was present in all normal tissues as well as in Ehrlich ascites tumor cells. These observations led us to compare the metabolism of P^{32} in phosphatido-peptide and other phosphorus-containing fractions between normal and tumor tissue. Consequently, the turnover rate of P^{32} in these fractions was determined *in vitro* using normal epidermis, "normal" epidermis from tumor-bearing mice and neoplastic epidermal tissue from mice.

Materials and methods. Male adult C57Bl/6 mice used in this study received laboratory chow and water *ad libitum*. The tumor, squamous cell carcinoma, was induced by topical painting with 20-methylcholanthrene to the dorsal unepilated interscapular skin daily for a period of 9 weeks as described previously(2). Histologically, the tumor showed the structure of an epidermoid carcinoma with occasional differentiation into spinous cells, keratinization and formation of epithelial pearls. Papillomas appeared within 9 weeks and squamous cell carcinoma about 12 weeks following the last application. Normal epidermis and "normal" epidermis from tumor bearing mice were obtained from the ventral surface of the mouse. Epidermal cells were harvested by scraping the sheets of shaved skin which had been incubated previously with 0.02% ethylenediamine tetracetic acid (EDTA)(3) for one hour at 0-4°C. Tumor cells were obtained after mincing the tumor mass with scissors and incubating with 0.02% EDTA for one hour at 0-4°C. Necrotic areas were removed before mincing the tumor. Tissues were incubated in Yeast extract-Eagle medium-Lactalbumin hydrolysate-Peptone medium (YELP)(4) under aerobic conditions at 37°C in Warburg vessels. Each ml of incubation medium contained approximately 50 mg wet weight of tissue and 10 μC of P^{32}. After 2 hours' incubation, the tissue was separated into the different phosphorus-containing fractions as described by Huggins and Cohn(1). The source of P^{32} and methods for determination of its activity were reported previously(1). The phosphatido-peptide content is expressed as μg P per unit dry weight and also per average cell because the latter may be altered in the case of the 3 different types of tissues studied. Each experiment represents the pooled tissues, epidermis or tumor, from 6 mice. The data in Table I and Fig. 1 represent the average of 4 experiments.

Results. Table I compares total phosphorus and P^{32} incorporation into phosphatido-peptide for normal epidermis, "normal" epidermis from tumor-bearing mice and neoplastic epidermal tissue. There was no apparent change in phosphatido-peptide phosphorus content of the tissue tested, in contrast to the marked stimulation of turnover of the phosphoryl radical as evidenced by the higher levels of specific activity both in neoplastic tissue and "normal" epidermis from the tumor-bearing mice when compared with normal epidermis.

Fig. 1 shows that not only is the specific

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TABLE I. Incorporation of P^{32} into Phosphatido-Peptides of Normal and Neoplastic Epidermis of Mouse Skin under *In Vitro* Conditions.

Tissue	Phosphatido-peptide content		Specific activity, cpm/μg P
	μg P/100 mg dry wt	μg P/cell $\times 10^{-7}$	
Normal epidermis	16.5	3.9	56
Epidermis from tumor-bearing mice	15	4.3	2511
Neoplastic epidermis (squamous-cell carcinoma)	10	4.2	2460

activity of the phosphatido-peptide phosphorus elevated in neoplastic epidermis and "normal" epidermis from tumor-bearing animals but also that these 2 tissues show a similar pattern for phospholipid, phosphoprotein and nucleic acid fractions. Of special interest, the specific activity of the nucleic acid phosphorus is higher in the "normal" epidermis from tumor-bearing mice after 2 hours of incubation than that found in the tumor. A similar pattern of incorporation of P^{32} *in vivo* was found in these 3 tissues; however, the differences between normal epidermis and the other 2 tissues were not nearly so great. A more detailed report including studies on the nucleic acid, phospholipid and phosphoprotein fractions of these tissues is in preparation.

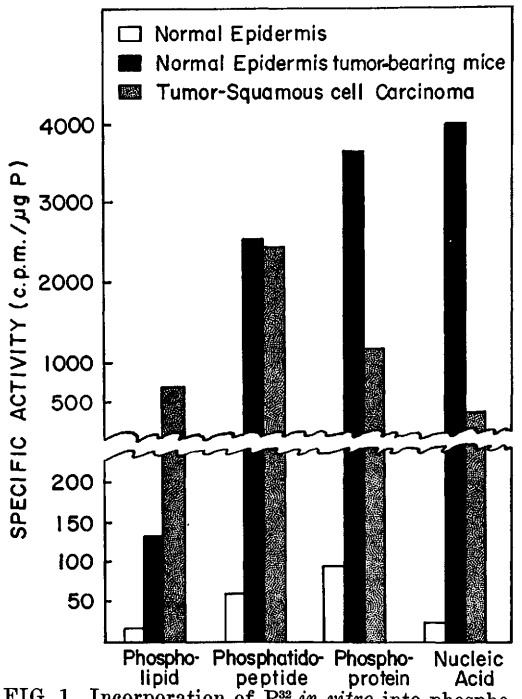

FIG. 1. Incorporation of P^{32} *in vitro* into phospholipid, phosphatido-peptide, phosphoprotein and nucleic acid fractions of normal epidermis, "normal" epidermis from tumor-bearing mice and neoplastic mouse epidermis. Specific activity is expressed as cpm/μg P. Aerobic incubation was for 2 hr in a medium containing about 30 mg wet wt of tissue and 10 μC of P^{32}/ml.

Discussion. In an investigation of the comparative results of phospholipid turnover in normal epidermis and a transplantable squamous cell carcinoma, Costello *et al.*(5) were not able to show a difference in specific activity of the 2 tissues *in vivo* using P^{32}. However, total activity was shown to be higher in the tumor due to an elevated phospholipid phosphorus content. Since our data were obtained using tumor tissue that was induced by painting with 20-methylcholanthrene the data from the 2 studies are not strictly comparable. Yet the turnover rate of phosphorus in phosphatido-peptide was much greater in tumor tissue than the corresponding normal tissue. We believe the effects reported are due to growing tumor since no effect was observed in mice painted with 20-methylcholanthrene until after the appearance of papillomas. In addition, there was no effect due to topical treatment with benzene, the solvent for 20-methylcholanthrene. These data indicated that a metabolic characteristic of neoplastic tissue can be demonstrated in histologically normal epidermis from animals with squamous cell carcinoma. It is of interest that Clark *et al.* (6) reported recently that premalignant and malignant lesions of the human vulva preferentially take up P^{32}.

Summary. Evidence was presented which demonstrates that the turnover of phosphorus in phosphatido-peptide is greater for neoplastic epidermis than normal epidermis. Histologically "normal" epidermis obtained from mice bearing a squamous cell carcinoma induced with 20-methylcholanthrene had a

phosphorus turnover more closely resembling neoplastic than normal tissue. Further studies concerning the biochemical changes mentioned are in progress.

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Effect of Bile Acids on Serum Cholesterol in the Chick.*† (26741)

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Recently Beher *et al.*(1) and Howe *et al.* (2) have reported that several bile acids (hyodeoxycholic acid, chenodeoxycholic acid and lithocholic acid) counteract the hypercholesterolemic effect of cholic acid in mice and prevent cholesterol accumulation in the liver and carcass. Portman and Bruno(3) have reported similar findings with rats. Experimental work on the effect of cholic acid or any other bile acids on serum cholesterol levels in chickens has not been carried out. Since the chicken is known to secrete quite different bile acids than the mouse and rat, it was desirable to investigate the effect of feeding various bile acids on serum cholesterol in chickens.

Experimental. The experiment was conducted with day-old cross-bred cockerel chicks (Cornish male X White Plymouth Rock female) obtained from a local hatchery. They were housed in electrically heated batteries with wire floors and were supplied feed and water *ad libitum*. They received the experimental diets for 3 weeks and were weighed at weekly intervals during this period. At termination of the growth phase of the experiment, blood samples were obtained by heart puncture and the serum separated for total cholesterol determination by the method of Schoenheimer and Sperry(4). The cholic acid, lithocholic acid and hyodeoxycholic acid used in the experiment were obtained from Nutritional Biochemicals Corp. These bile acids were added to the diet at the 0.2% level singularly and combinations of cholic and lithocholic acids, and cholic and hyodeoxycholic acids. The basal diet used was similar to that used in earlier work(4), modified to contain 4.0% corn oil. All substitutions in the diet were made at the expense of fiber.

Three groups of 8 chicks each were started on each of the dietary treatments. The results were analyzed for statistical differences by the Multiple Range Test(5).

Results. The effect of feeding the various bile acids on growth and serum cholesterol is shown in Table I. Addition of lithocholic acid to the basal diet caused a marked depression in growth rate. When cholic acid was added to the diet containing lithocholic acid this retardation in growth rate was reversed. Addition of cholic acid, hyodeoxycholic acid or a combination of these 2 bile acids to the basal diet had no statistically significant effect on growth rate of cockerels. However, it should be noted that rate of growth was faster when both hyodeoxycholic acid and the combination of hyodeoxycholic acid and cholic acid were added to the basal diet.

While lithocholic acid depressed growth, it caused an approximately 3-fold increase in serum cholesterol level of the cockerels. Addition of cholic acid to the diet containing

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