process. Also, Howard *et al.*(11) observed that increased metabolic activity of cells of the reticulo-endothelial system of BCG immunized animals may bring about development of intracellular environment unfavorable for bacterial multiplication. Should these cellular alterations function to their fullest extent, availability of immune monocytes in localized areas of infection could be sufficient to confine and inhibit the tuberculous process.

Summary. Experiments are reported which demonstrate passive transfer of a limited degree of increased resistance to tuberculosis through use of monocytes from immunized animals. Use of monocytes from normal animals had no effect on resistance. No increased resistance was seen in animals receiving plasma, spleen homogenate, or spleen filtrate obtained from either normal or immunized animals.

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Chromatography of Proteins of Squamous Cell Carcinomas and Normal Epithelium of Mice.* (25507)

PIETRO U. ANGELETTI, BLAKE W. MOORE, STOJAN SOLARIC, VALENTINA SUNTZEFF
(Introduced by E. W. Dempsey)
Wernse Cancer Research Laboratory, Washington University School of Medicine, St. Louis, Mo.

Electrophoresis has been used to fractionate proteins of extracts of various tumors and normal tissues(1,2). The method of chromatography of proteins on substituted cellulose ion exchange columns has been used in this laboratory to fractionate soluble proteins of liver and several enzyme activities localized in various fractions(3). The present paper is concerned with chromatographic fractionation of proteins of extracts from squamous cell carcinomas and normal epidermis of mice. Three enzymes have been localized in the fractions.

Methods. The normal epidermis of mice was removed from dermis mechanically as reported previously(4). Tumors used were: (a) primary squamous cell carcinomas induced in Swiss mice by painting the skin with methylcholanthrene; and (b) 3 lines of transplanted squamous cell carcinomas carried for several generations in this laboratory. Extracts were prepared by homogenizing at 0°C at high speed with a "Virtis" homogenizer in .005 *M* tris (hydroxymethyl)aminomethane-phosphate buffer, pH 8.00 ± 0.03. The homogenates were then spun 30 to 45 minutes at 13,500 rpm in Sorvall SS-1 centrifuge; the clear supernatants were dialyzed overnight against the same buffer, then stored frozen at −15°C. Chromatography on diethylaminoethylcellulose anion-exchange columns was carried out as reported previously(3). These methods will be reported later. Proteins were determined by the Lowry method(5); in addition absorbances at 280, 260 and 413 mμ were measured in each fraction. For β-glucuronidase assay, the Fishman method was used(6); for lactic-dehydrogenase (LDH),

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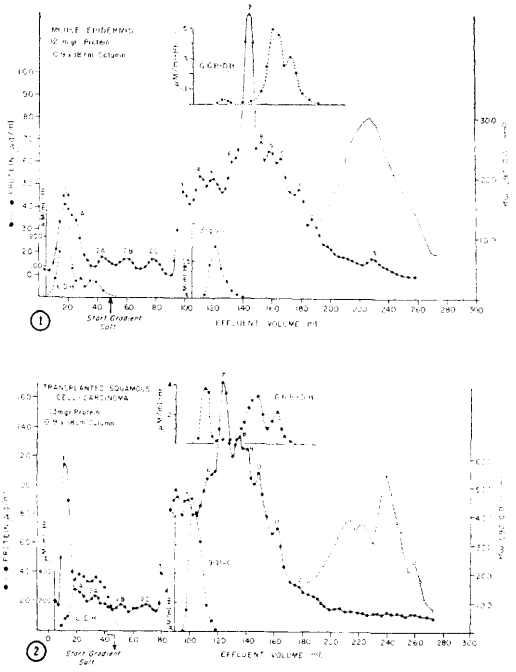

FIG. 1. Typical chromatogram of extract of normal epidermis on DEAE-SF.

FIG. 2. Typical chromatogram of extract of squamous cell carcinomas on DEAE-SF.

the Kornberg method(7); and for glucose-6-phosphate dehydrogenase (G-6-PDH), the Buell, *et al.*, method was used(8). Six experiments with extracts of normal epidermis, and 4 with extracts of each type of tumor were carried out.

Results. In all experiments the protein patterns showed some similarity (Fig. 1,2), the various peaks appearing approximately at the same chloride concentrations during chromatography of either normal or cancer tissue. The material in peaks 1 through 12 showed typical protein ultraviolet absorption, while that appearing between 0.3 and 0.5 *M* NaCl had maximum absorbance at 260 mμ. Hemoglobin, evidenced by 413 mμ absorbing material, appeared in all chromatograms in 2 or more fused peaks between 0.05 and 0.07 *M* NaCl. Nucleic acids (260 mμ absorbing material) appeared in only one sharp peak in all chromatograms of normal epidermis extracts; in chromatograms of all tumor preparations they appeared in a wider zone with 2 major peaks (Fig. 1,2). Whether this is related to a different quantitative or qualitative composition of nucleic acids in the extracts of tumors and normal epidermis requires further investigation. The graphs also show distributions of 3 enzyme activities; other chromatograms gave the same patterns with some variations in relative peak heights. LDH activity in chromatograms of normal epidermis appeared as one sharp peak corresponding to the first protein peak, followed by at least 2 smaller peaks. In all tumor chromatograms, LDH activity was present in a wider zone localized in a region following first protein peak and was shifted to the right as compared with its position in normal chromatograms (Fig. 2).

β-glucuronidase activity was localized in the same place in all chromatograms, with one sharp peak which was higher in extracts of tumors than in those of normal epidermis. Glucose-6-phosphate dehydrogenase activity showed 3 distinct peaks in tumor chromatograms (Fig. 2): however, in the chromatograms of extracts of normal epidermis there was evidence of only the 2 more tightly bound peaks, the first one extremely low or absent. Appearance of this first high peak of activity in tumor preparations can be correlated with the reported observation of increase of glucose-6-phosphate dehydrogenase activity in neoplastic liver homogenates(9).

Localization of these 3 enzymes in the chromatograms of tumors as well as in normal epi-

TABLE I. Ratio between Chloride Concentration Required to Elute the Various Peaks of Enzyme Activities and Chloride Concentration Required to Elute the Nucleic Acid Peak.

		(Cl^-) enzyme peak/(Cl^-) N.A. peak			
		G-6-PDH			
Tissue	No. exp.	Peak 1	Peak 2	Peak 3	β-glucuronidase
Liver	3	.20	.40	.48	.15
Epidermis	5		.41	"	"
Transplanted tumors	9	"	.40	"	"
Induced tumors	4	"	"	.47	"

dermis was comparable to that found during chromatography of mouse liver extract by the same procedure. When the positions of various peaks of activity were expressed relative to chloride concentration required to elute the major peak of nucleic acids (Table I), the β-glucuronidase activity appeared in each chromatogram in the same relative position.

In liver extracts glucose-6-phosphate dehydrogenase activity appeared as 3 peaks (Table I). Each peak of activity of this enzyme in tumor and epithelium chromatograms corresponded to one of the 3 of liver.

Summary. These results show that the above chromatographic procedure can be used to separate different protein extracts into a number of fractions and to investigate the distribution of various enzyme activities to pick out qualitative enzymic differences between malignant tumors and the normal tissues from which they arise.

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Free Phenolic Substances in Blood Plasma.*† (25508)

JOHN W. GREENE, JR. AND JOSEPH C. TOUCHSTONE
(Introduced by F. D. W. Lukens)
Dept. of Obstetrics and Gynecology, School of Medicine, University of Pennsylvania, Philadelphia

Veldhuis(1), Nakao and Aizawa(2), Aitken and Preedy(3), Slaunwhite and Sandberg(4), and Oertel *et al.*(5) have described chemical methods for determination of estrogens in blood. The methods of Nakao and Aizawa, and Aitken and Preedy employ column chromatography for final separation of the estrogens prior to estimation by fluorometry. In our laboratory an alumina column similar to that described by Nakao and Aizawa was employed. However, instead of eluting the column with 1% ethanol in benzene for the estrone fraction, elution was carried out with 0.25, 0.50 and then 1.0% ethanol in benzene. When this was done, it was found that the free "estrone" fraction actually consisted of a substance slightly less polar than estrone. This phenolic substance less polar than estrone would be estimated as estrone if elution were performed with only 1.0% ethanol in benzene. The following is a description of results of estimations of free estrone and estradiol-17β fractions in plasma. Some properties of a substance less polar than estrone are also described.

Method. Heparinized blood was centrifuged immediately after withdrawal and the plasma separated. The method of Brown (6) was adapted for extraction. Plasma (20 ml) was extracted by partition once with 4 volumes of ether for 30 min. and then twice with 2 volumes for another 15 min. The ether extract was washed with 16 ml of concentrated sodium carbonate at pH 10.5 and partitioned with 4 ml of 2 N sodium hydroxide. The sodium hydroxide was adjusted to pH 10.5 by addition of 16 ml of 8% sodium bicarbonate,

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