42.5°C, showed cells in an alveolar arrangement similar to that in the tumors grown at 37.5°C, but there was a complete absence of any sarcomatous component, and the character of individual tumor cells differed (Fig. 1*c*). There were many giant cells at 42.5°C, with bizarre nuclei in which mitotic figures could be found in some instances.

Evidently, when the eggs were incubated at 32.5°C, the sarcomatous potentialities of the tumor became dominant. The differing patterns of tumor growth may be the result of a cytogenetic selection in which certain cells, either with sarcomatous or with carcinomatous potentialities, are selected favorably at different temperatures. Alternatively, differing characters of growth may be examples of modulation. The same stem cells may, under one set of circumstances, grow predominantly in a spindle-shaped pattern, and under another, in an epithelial pattern (*3*).

JOSEPH LEIGHTON
LEONARD MERKOW*
GERALD JUSTH
Department of Pathology, School of Medicine, University of Pittsburgh Pittsburgh 13, Pennsylvania

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* Sarah Mellon Scaife research fellow in pathology.

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Chromatographic Heterogeneity of Rabbit Serum Amylase

Abstract. *A chromatograph of normal rabbit serum shows three major peaks of amylase activity. Material associated with each peak differed in composition of serum protein, electrophoretic distribution of amylolytic activity, optimum* p*H for activity and Michaelis-Menten constants for starch hydrolysis. These data suggest but do not prove conclusively that normal rabbit serum contains at least three molecular forms of amylase.*

At least two amylases have thus far been distinguished in the serum of man and a variety of experimental animals by electrophoretic (*1–3*) or immunologic techniques (*4*). These observations suggest that the amylase activity of normal serum represents the net action of a group of amylolytic enzymes which may be elaborated by a number of different tissues. To elucidate the heterogeneity of serum amylase and the physico-chemical properties of these amylases, we have chromatographically separated fractions of normal rabbit serum and characterized the separate fractions in terms of electrophoretic behavior and enzyme activities.

Samples (approximately 3.0 ml) of serum from five normal rabbits were treated with Sephadex G-25 to remove the ionizing salts. The samples were then applied to DEAE-cellulose (diethylaminoethyl) columns and eluted with a chloride gradient solution at a flow rate of 20 to 25 ml per hour. The effluent showed three major peaks of amylase activity (Fig. 1). Peak 1 (tubes 10 to 20) was neither bound by DEAE-cellulose, nor associated with appreciable quantities of serum protein. With increasing chloride, two additional well-defined maxima of saccharogenic activity were observed in effluent tubes 30 to 45 (peak 2) and 46 to 80 (peak 3). Tubes 30 to 80 also contained protein. The chromatographic behavior of peak 1 suggests that the material is less negatively charged than that of peaks 2 or 3. Differences in charge may also account for the appreciably greater quantities of protein in the effluents from peaks 2 and 3.

The mean percentage distribution of amylase activity in peaks 1, 2, and 3 of the five rabbit sera were 26.4, 31.5, and 20.4 percent of total activity, respectively. The total recovery of amylase activity in no case exceeded that of unfractionated rabbit serum. Similar results were noted in electrophoretically separated fractions of rat serum as compared with whole rat serum (*5*). By contrast, the amylase activity recovered from normal human serum subjected to paper electrophoresis totaled more than the activity recorded in the unpartitioned serum (*1*, *2*). This discrepancy may be due to an amylase inhibitor which is active in whole serum (*1*).

Collections of effluent from each peak were pooled, dialyzed for 24 hours against distilled water, lyophilized, and reconstituted in 0.5 ml distilled water. Samples of the concentrate (20 μl) were placed on Whatman 3MM filter paper and subjected to electrophoresis for 16 hours in a horizontal chamber at a constant potential of 80 volts in veronal buffer (*p*H 8.6; ionic strength, 0.075). When the strips were stained with bromphenol blue, the protein in

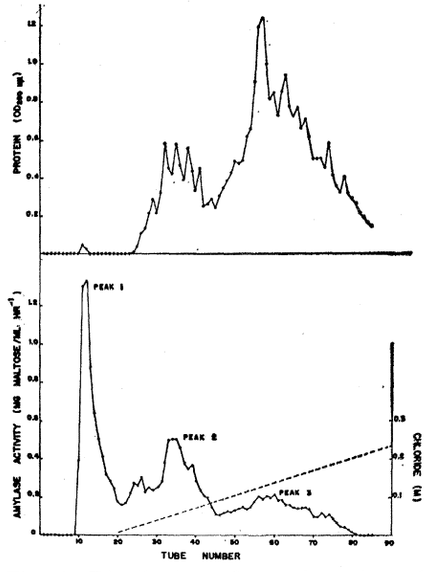

Fig. 1. Distribution of protein estimated spectrophotometrically (*9*) (top), and saccharogenic activity (*10*) (bottom) of normal rabbit serum fractionated by means of a chloride gradient on a DEAE-cellulose column (30 × 1.5 cm, Whatman paper DE-50 (*11*).

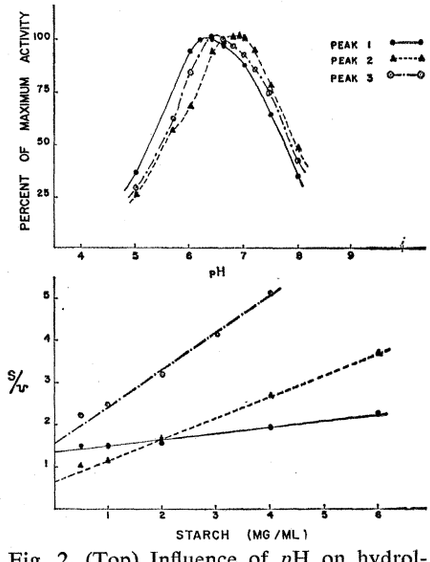

Fig. 2. (Top) Influence of *p*H on hydrolysis of starch by chromatographic fractions of normal rabbit serum. (Bottom) Relationships between starch substrate (*s*) concentrations and reaction speeds (*v*) used to calculate *km* values of 4.4, 0.6, and 0.9 mg/ml for peak 1, 2, and 3 concentrates, respectively.

the concentrate from peak 2 had electrophoretic mobilities similar to γ- and β-globulin of whole rabbit serum. Most of the material appeared to be γ-globulin, with much lesser amounts of β-globulin. In contrast, electrophoretic patterns obtained from peak 3 concentrate appeared to contain all the protein fractions in whole rabbit serum except the γ-globulin. These data suggest that certain amylase fractions are separated chromatographically with particular serum proteins.

Strips prepared from the electrophoresis of 20 μl samples of the peak concentrates were also incubated on blocks of starch and agar gel for 24 hours at 37°C (*6*). After removing the paper strips, the blocks were flooded with 0.1*N* iodine solution. Absence of the starch-iodine reaction indicated areas where starch was hydrolyzed during incubation. When treated in this manner, the material of peak 1 concentrate showed a single pronounced zone of starch hydrolysis on the cathodal side adjacent to the point of application. A similar distinct zone of amylolytic activity on the anodal side was observed with strips containing peak 2 concentrate. The opposite electrophoretic mobilities of amylase activity in peak 1 and 2 concentrates may be attributable to differences in charge of the molecules. These results are consistent with the chromatographic behavior of the fractions.

The starch-hydrolysis pattern yielded by peak 3 concentrate revealed three bands of enzymatic activity. These corresponded respectively to albumin, the zone between α_2- and β-globulin, and γ-globulin. Activity corresponding to the position of γ-globulin was of interest because paper electropherograms obtained from this concentrate failed to show stainable γ-globulin. The principal zones of hydrolysis corresponded to the electrophoretic positions of albumin and γ-globulin, whereas much smaller amounts of starch were split in the region between the α_2- and β-globulin zones. Possibly, the heterogeneity of peak 3 concentrate can be partially attributed to incomplete chromatographic separation.

The amylolytic activities of the three peak concentrates were measured in citrate and phosphate buffers (*p*H from 5 to 8), with Lintner starch as substrate. The optimum *p*H for starch hydrolysis of peak concentrates 1, 2, and 3 was 6.2, 6.9, and 6.6, respectively (Fig. 2, top).

Lintner soluble starch in phosphate buffer (0.1*M*, *p*H 6.9) was used to estimate Michaelis-Menten constants (*Km*) for the three peak concentrates at 37°C (Fig. 2, bottom). The enzymatic material in peak 1 concentrate yielded the largest *Km* (4.4 mg of starch per milliliter). The smallest value for the constant (0.6 mg of starch per milliliter) was observed with peak 2 concentrate. The *Km* value for peak 3 (0.9 mg of starch per milliliter) was intermediate between the other two constants.

Amylolytic activity of peak 1 concentrate was enhanced at a lower *p*H and exhibited a larger *Km* than the other chromatographic fractions. It is possible that the presence of protein in peak 2 and 3 concentrates modified the relationship of enzyme activity to *p*H and the *Km* values. Although a number of nonprotein substances have been shown to alter amylolytic activities of hog pancreatic amylase (*7*), the influence of serum proteins on starch hydrolysis remains to be established.

Thus rabbit serum seems to contain several amylases that can be distinguished chromatographically (*8*).

J. E. BERK
Department of Medicine, California College of Medicine, Los Angeles
M. KAWAGUCHI
R. ZEINEH
Department of Medicine, Sinai Hospital, Detroit, Michigan
I. UJIHIRA
R. SEARCY
Department of Medicine, California College of Medicine

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Thermally Induced Genital Appendages on Mosquitoes

Abstract. *Temperatures above those normally present in larval sites of certain mosquitoes (for example,* Aedes stimulans*) cause larvae of potential males to be feminized in all degrees. Certain combinations of temperatures will affect these larvae so that an abnormal pair of genital appendages consistently appears in addition to the usual pair. These appendages appear to be masculine and are presumed to come from imaginal discs that are latent in present-day Diptera.*

*

In connection with a more inclusive study of the effects of thermal stress on anomalous development of subarctic aedine mosquitoes heretofore unknown, genital appendages have been produced on *Aedes stimulans* (Fig. 1) and other species. These appendages appear on each side as paired expansions from the caudal part of the eighth abdominal segment (Figs. 1 and 2, bottom left). They develop anterior to and in conjunction with the normal male genitalia and are, in no sense, replacements. This anomalous pair is produced consistently in response to controlled temperatures such as are not encountered normally during larval life. This pair does not appear on the eighth segment when larvae are reared at temperatures that are normal for the species (Fig. 2, top left).

Aedes stimulans is a floodwater mosquito which normally develops through four larval instars only in cold water (5° to 20°C). Under such conditions, the adult population is sexually dimorphic (*1*). Half of the larval population is limited to becoming females because it is homozygous for female traits. The other half is capable of becoming either sex or admixtures of both sexes because it is heterozygous for sex. The heterozygous component becomes wholly male only when reared at temperatures less than 23°C. It becomes female when reared at temperatures above 28°C. Each dimorphic part (antennae, palpi, genitalia, gonads, and so forth) may be partially or wholly feminized within this range of temperatures (*1*, *2*). When maleness is inhibited thermally, elements for femaleness express themselves as is the case also with at least 12 other subarctic species.

When larvae are reared at different combinations of temperatures, wholly