F_{12}. Bei Fällung von F_{11} mit mehr Aceton (73,5 ml) verringerte sich wohl das γ-Globulin in der Fraktion F_{12}, dafür fand sich aber Albumin in der Fraktion F_{11}, und auch der Anteil der α- und β-Globuline war erhöht.

In den Figuren 3 und 4 sind die durch fraktionierte Acetonfällung erhaltenen Fraktionen F_1, F_2, F_{11} und F_{12} aus Affen(*Macacus rhesus*)- und Meerschweinchenserum dargestellt. Nur durch Änderung der für die Fällung der Fraktionen F_1 und F_{11} notwendigen Acetonmenge gelang es in diesen beiden Fällen nicht, eine weitgehend gereinigte γ-Globulinfraktion zu erhalten.

Unter konstanten Bedingungen (pH, Ionenstärke und Ionenmilieu) gelang es, aus Kaninchenserum, hingegen nicht aus Affen- und Meerschweinchenserum, ein weitgehend gereinigtes γ-Globulin zu erhalten. Eine weitere Reinigung der γ-Globuline durch Änderung von pH, Ionenstärke und Ionenmilieu ist in Vorbereitung.

Summary. It is possible — under constant conditions, such as pH, ionic strength and ionic milieu — to purify γ-globulins from rabbit-serum, using the fractionated acetone precipitation.

TH. MESTER[8]

Stiftung zur Erforschung der spinalen Kinderlähmung und der Multiplen Sklerose, Hamburg-Eppendorf, 16. Dezember 1960.

[8] Mit technischer Assistenz von Fräulein CHRISTEL BORNA.

Pinguinain: Comparison of Cuban, Puerto Rican and the Thermal Properties

Introduction. The existence of two varieties of Bromelia pinguin fruit was observed by ASENJO and FERNANDEZ[1]. A long, thin fruit that gives a poor yield of juice is found in sandy soils near the seashore, and a smaller, round, thick fruit that yields large quantities of juice, grows in the interior at higher elevations. These variety differences have been noted in our laboratories in shipments received from Cuba and Puerto Rico. The fruit received from Cuba was long and thin and that from Puerto Rico was of the round, thick variety. ASENJO and FERNANDEZ[1] chose the latter fruit for their work; whereas, we performed our previous studies[2,3] as well as the present thermal studies on the Cuban variety.

In order to determine whether the proteins responsible for the enzymatic activity of these fruits were similar, electrophoretic study with the subsequent construction of electrophoretic titration curves for the components was employed.

Experimental. Electrophoretic Comparison: Samples of crude preparations[3] as well as purified fractions[3] from both the Cuban and Puerto Rican varieties were subjected to analytical electrophoresis in a Perkin-Elmer Model 38A apparatus. A variety of ionic strengths from 0.05 to 0.15 and of pH conditions from 3.5 to 6.6 were used with various preparations to determine the number of components and the mobilities of these components in both the Cuban and the Puerto Rican fruit.

Buffers of 0.1 ionic strength were used in the determination of mobilities for the electrophoretic titration curves. The buffers employed below pH 4.0 were glycine HCl–NaCl. From pH 4.5 through 5.35 the buffers were acetate. Between pH 5.5 and 5.85, both acetate and phosphate NaCl buffers were utilized while above pH 5.85 only phosphate NaCl buffers were used.

Thermal Studies: The proteolytic activities of the crude Cuban pinguinain were determined by a modification[3] of the BIDWELL[4] and OAKLEY[5] azocoll procedure.

To determine the thermal inactivation of pinguinain, 1 ml aliquots of enzyme in acetate, pH 5.2, buffer were incubated in a water bath at the given temperatures for 10 min. The solutions were immediately diluted to 10 ml with ice cold acetate buffer containing enough cysteine to give a final concentration of 0.05 *M*. The protease activity was then determined at 37°C.

The effect of temperature upon the reaction rate was determined in acetate buffer, pH 5.2, containing 0.05 *M* cysteine. In this study the enzyme was incubated for 20 min in the presence of the substrate at the given temperatures.

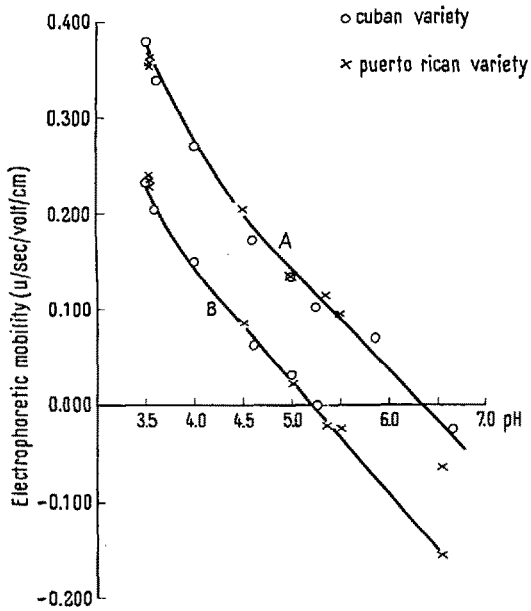

Fig. 1. Electrophoretic titration curve for pinguinain A and B in 0.1 ionic strength buffers

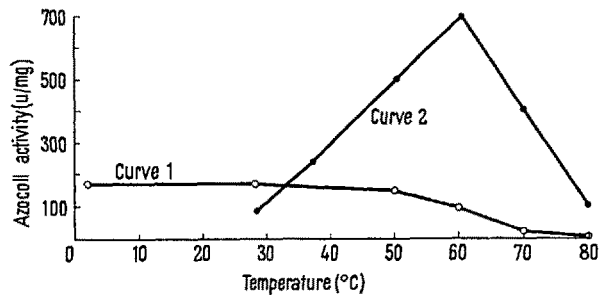

Fig. 2. Effect of temperature on the reaction rate and thermal inactivation of pinguinain solutions

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Results and Discussion. Electrophoretic Comparison: Under all conditions of ionic strength and of pH, no more than two mobile electrophoretic components were observed with both the Cuban and Puerto Rican preparations. When the analyses were performed under similar conditions of pH and of ionic strengths, preparations from both varieties of fruit exhibited the same number of components and their mobilities fell within experimental error on each other.

The 0.1 ionic strength electrophoretic titration curves (Fig. 1) for the Puerto Rican pinguinain components are superimposable upon those of the Cuban proteins. For both varieties the isoelectric point for pinguinain A is approximately pH 5.2, and for the B-component the isoelectric pH is 6.4. Since the two protein components of these fruits behave identically when analyzed electrophoretically, the probability is great that they are the same proteins and that they perform the same enzymic functions.

Thermal Studies: Figure 2, curve 1 shows the effect of heat upon the activity of enzyme solutions exposed to various temperatures for 10 min in the absence of the substrate. Above 50°C activity decreased rapidly with almost complete inactivation occurring at 80°C.

The effect of incubation temperatures upon reaction rate is recorded in curve 2 of Figure 2. For each 10°C rise in temperature up to 60°C, activity increased approximately 200 μ/mg, while above 60°C activity decreased approximately 300 μ/mg. The 20 min temperature coefficients, Q_{10}, for the crude pinguinain-azocoll reaction are as follows: $Q_{10}(30°–40°C) = 2.40$; $Q_{10}(40°–50°C) = 1.66$; $Q_{10}(50°–60C°) = 1.40$.

Zusammenfassung. 1. Pinguinain-Präparate aus zwei verschiedenen Bromelia-Pinguinen enthielten dieselbe Anzahl elektrophoretischer Eiweisskomponenten von ähnlicher Laufgeschwindigkeit bei verschiedenen Ionenstärken und pH-Puffer-Bedingungen. 2. Pinguinain-Lösungen, die bei Temperaturen über 50°C gehalten wurden, zeigten starken Aktivitätsabfall. 3. Das 20-min-Temperatur-Optimum für die Roh-Pinguinain-Azokollreaktion wurde bei ca. 60°C gefunden. Es werden Q_{10}-Werte für die Roh-Pinguinain-Azokollreaktion angegeben.

R. A. MESSING

Biochemistry Department, Research Division, Ethicon Inc., Somerville (N. J.), November 14, 1960.

Endomeiosis in Parthenogenetic Lines of Aphids

It is generally admitted that the parthenogenetic egg of Aphids undergoes a single maturation division and a single diploid polar body is formed. It is believed therefore that chromosomes divide as in a normal mitosis and the eggs consequently develop with a diploid chromosome set that has not undergone pairing[1–3]. For this reason parthenogenesis in Aphids has been classified in the ameiotic type[4,5], although a transient pairing of chromosomes has been observed in three species of Aphids by VON BAEHR[6] and PASPALEFF[7]. No genetic variability should therefore be present in single parthenogenetic lines of Aphids.

Parthenogenetic strains of *Myzodes persicae*, which were originated from a single female, were employed for selection experiments, and the number of winged forms was reduced until they disappeared altogether in the course of nine generations. Selection was carried on through elimination of winged individuals in environmental conditions that appeared to be clearly favourable to the appearance of winged individuals in the controls[8].

The demonstration of genetic variability showed therefore the necessity of a cytological reinvestigation of the maturation divisions of the parthenogenetic eggs in Aphids. Research was carried on in *Macrosiphum rosae*, *Myzodes persicae*, and *Brevicoryne brassicae* which resulted to present the diploid chromosome number of 10, 14, 16 respectively.

Thin chromatic filaments that show granules of varying thickness, which can be interpreted as chromomeres, can be observed in the nucleus of oocytes of very young embryos. Leptotene and zygotene stages could not be distinguished exactly (Fig. 1a). The filaments undergo successively a remarkable contraction and the parallel arrangement of the homologous chromosomes in the haploid number of bivalents becomes evident. The homologous granules of the filaments are also well distinguishable (Fig. 1b). This stage is interpreted as a pachytene stage.

Each bivalent contracts until it assumes a thick irregularly rhomboidal form which shows an empty space in the middle. The nucleolus is always present (Fig. 1c). This stage is considered as intermediate between diplotene and diakinesis stage.

Chromosomes of *M. rosae* and *B. brassicae* appear highly contracted into two pairs of strictly linked hemispheres when the oocyte begins the descent into the ovarian chamber (Fig. 1d; 2). The single bivalents then divide and the univalents remain within the nucleus because no achromatic spindle is formed and the nuclear membrane is not dissolved. The detachment is not synchronous in all the bivalents and the univalents can be observed when some chromosomes are still paired (Fig. 1e; 3). When all the bivalents have separated, 10 chromosomes can be counted in *M. rosae*, 16 in *B. brassicae* within the nuclear membrane.

Chromosomes of *M. persicae* do not assume the appearence of paired hemisphere, although they continue to contract after diakinesis stage. The detachment of bivalents is also asynchronous and the univalents remain within the nucleus (Fig. 1d, e).

The nucleolus of *M. rosae* disappears before the oocyte has passed into the ovarian chamber. It is always present in other species.

After the bivalents have separated and the diploid number of chromosomes has been restored, the chromosomes concentrate in the centre of the nucleus in *M. rosae*, they remain scattered in *B. brassicae* and homologous chromosomes tend to approach each other in *M. persicae*.

Chromosomes then become less distinct and the nucleus shows filaments and scattered chromatin granules. In *M. persicae* chromosomes condense around the nucleolus. The oocyte increase considerably in size, big vacuoles are

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