THE MILK COMPOSITION OF ROCKY MOUNTAIN BIGHORN SHEEP (OVIS CANADENSIS CANADENSIS)

BY

ERNEST CHUNG-HSU CHEN

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ABSTRACT

Milk was collected from five Rocky Mountain bighorn sheep living in the Jasper National Park area of Alberta. The milk was collected at 1 1/2 and 3 months post partum. The total solids, ash, fat, lactose and protein contents of the five samples were determined. The fatty acids constitution of the milk fat was estimated by gas-liquid chromatography. The electrophoretical behaviours of the various caseins and whey protein were studied in both starch and polyacrylamide gel medium. The hexose, hexosamine and sialic acid contents of the casein samples were determined spectrophotometrically. For the purpose of comparison, a sample of domestic sheep milk (Suffolk, 1 month post partum) was investigated in exactly the same manner as were the bighorn sheep milk samples.

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I. General Introduction

The Rocky Mountain bighorn sheep is one of the important native ungulates in Canada. The total population of the animal was estimated, early in 1944, to be 2259 in Jasper National Park of Alberta alone (Cowan, 1944) and the number has been increasing progressively since 1944.

The Canadian Wildlife Service, Department of Northern Affairs and National Resources has initiated a study of the biology and ecology of the bighorn sheep which inhabit the Mountain Park Regions of Canada. A study of the composition of the milk of these animals has been included in this proposed work. The results of this study will (a) make possible a comparison of the milk of the bighorn sheep with that of other ungulates and with domestic animals and (b) provide essential information in connection with the early growth and viability of the bighorn lambs.

The present thesis deals with (a) the gross composition of bighorn sheep milk; (b) the fatty acid constitution of bighorn sheep milk fat; (c) the electrophoretic analysis of sheep milk casein and whey protein and (d) the carbohydrate (hexose, hexosamine and sialic acid) content of sheep milk casein.

II. Literature Review

II. 1. Bighorn Sheep and Sheep Milk Analysis

Bighorn sheep is one of the native sheep of North America. It is found in the Rocky Mountains from Wapiti Pass in the North to Utah and Colorado in the South (Cowan, 1940). In Canada, bighorn sheep are found on grassy slopes close to rough terrain in the area of Jasper and Banff National Parks, Alberta.

The subspecies of the bighorn sheep from which the milk samples were obtained for the present investigation, is Rocky Mountain bighorn sheep, Ovis canadensis canadensis Shaw. This animal belongs to same family Bovidae, subfamily Ovinae and genus Ovis Linnaeus as does domestic sheep (Hall and Kelson, 1959).

Ovis canadensis canadensis is a large sheep, with robust body (especially the male), slender legs, short tail, narrow nose, beardless chin and somewhat pointed well-haired ears. Both sexes carry horns with well-marked brownish transverse ridges from base to tip. The horns of adult males are massive, curving backward from the base, then outward, downward and

forward in a majestic sweep. The horns of adult females are small and comparatively slender, with an upward and backward sweep, not curled. Adult rams weigh between 200 and 300 pounds, and stand from 38 to 42 inches high at the shoulder. The ewes stand less than three feet high, and weigh between 125 and 175 pounds (Green, 1949). The general appearance of the animal is shown in Figure 1. (Downing, 1947).

Figure 1 Rocky Mountain Bighorn Sheep (Ovis canadensis canadensis Shaw)

The total population of bighorn sheep in Rocky Mountain area is not known. However, early in 1944, Cowan estimated

that the number of bighorn sheep (one year old and over) in Jasper National Park was 2259. In the same report, Cowan described twenty different bands of bighorn sheep in Jasper National Park. He pointed out that certain bands were fairly isolated and had little communication with other bands. However, there appeared to be considerable communication between some bands. Downing (1947) noted that adult rams tend to wander about in small groups of 5 or 6, while the ewes, lambs and immature males stay in groups of 20 to 30 animals.

Flook (1964) reported that grasslike plants, forbs and browse of mountain meadows make up the major diet of the animal. In winter, much of the food is secured by scraping away the snow from the vegetation beneath it.

In contrast to the voluminous literature on the composition of cow's milk, data on sheep's milk is comparatively sparse. The following authors have published the results of detailed analyses of sheep milk:- Godden and Puddy (1935); El-Sokkary et al. (1949); Jankowski (1953); Barnicoat et al. (1956); Prekopp (1957); Sartore and Lai (1957); Iotov and Ikonomov (1957); Perrin (1958); Nejjim (1963) and Ashton et al. (1964).

A search of the literature failed to locate any published data on the composition of the milk of Rocky Mountain bighorn sheep.

II. 2. Fatty Acid Composition of Milk Fat.

The triglycerides of milk fat probably contain a greater variety of acids than any other natural fat. Our knowledge of the composition of milk fat has increased greatly with the development of new analytical tools for fat analysis. One of the most outstanding contributions was gas-liquid chromatography made by James and Martin (1956) who applied the method to the analysis of goat milk fat. Since 1956 this technique has been used very extensively for the study of milk fat composition.

Craig and Murty (1958) showed that fatty acid methyl ester could be separated according to chain length by the use of washed silicone grease supported on firebrick. They reported that the same esters could be separated according to both chain length and degree of unsaturation when a plasticizer was used on the firebrick. Patton et al. (1960) determined butyrate independently by GLC employing an ionization detection system. Smith (1961) developed a procedure by which methyl esters of the fatty acids were prepared by methanolysis and then dissolved in ethyl chloride. The short-chain acid peaks were recorded on the GLC chart by increasing the chart speed.

DeMan (1961) devised a method by which the short-chain

fatty acids could be accurately determined. He salted out the methyl esters with saturated potassium bisulfate solution, then extracted them with pentane and resolved the short-chain peaks by operating the GLC column at low temperature(78° C). Because of the fact that butyl esters are much less volatile than methyl esters, Gander et al. (1962) esterified the short-chain fatty acids (C_4 - C_8) into butyl esters and resolved them independently on a nonpolar (Apiezon L) GLC column with temperature programming. The long-chain fatty acids were made into methyl esters and separated on a polar (diethylene glycol succinate) GLC column.

Jensen et al. (1962) used these improved GLC methods to determine the fatty acid composition of the lipid fraction of pooled raw milk. Table 1 shows the results of the fatty acid composition of butterfat obtained by several workers.

Bills et al. (1963) employed a GLC column packed with 20% diethylene glycol succinate supported on 80-100 mesh Celite 545 to determine the free fatty acid in milk fat. The free fatty acids were isolated from the fat by means of a basic ion-exchange resin (Amberlite IRA - 400). A special reflux system for concentration of the ethyl chloride extract was used to avoid the loss of volatile esters. Heptanoic and heptadecanoic acids were added to the extract prior to methylation, as internal standards. A temperature

of 94° C was employed for the C_4 - C_8 acids and 194° C for the C_{10} - C_{18} acids.

DeMan (1964) prevented the loss of the methylbutrate by carrying out the esterification process in a sealed bulb at 60° C. Satisfactory separation of the short-chain fatty acids was achieved by using a dual-column temperature-programmed gas chromatograph.

Table I.
Fatty Acid Composition of Bovine Milk Fat
as Determined by GLC Method (Weight %)

Components	Analysis of			
	Samuels et al. (1960)	Patton** et al. (1960)	Gander et al. (1962)	Jensen et al. (1962)
4:0 *	1.1	3.6	3.6	3.57
6:0	1.7	I.4	2.3	2.22
8:0	1.4	1.0	1.2	1.17
9:0	---	0.01	0.1	0.03
10:0	3.0	2.6	2.8	2.54
11:0	0.4	0.04	0.4	0.33
12:0	3.7	3.3	2.9	2.81
12:1	---	---	0.4	0.33
13:0	T	0.08		
14:0 iso	T	---	0.2	0.17
14:0	11.9	11.3	10.0	10.06
14:1	1.6	---	1.9	1.63
15:0 iso	---	---		
15:0	1.6	1.1	1.1	I.09
15:1	---	---	0.4	---
16:0 iso	0.4	---		0.38
16:0	31.0	29.9	24.8	24.97
16:1	3.0	1.1	2.6	2.55
17:0	0.8	0.7	1.1	0.91
17:1	---	---	0.3	---
18:0 iso	---	---		0.38
18:0	11.2	13.7	11.9	12.07
18:1	24.0	*** 17.9	27.1	27.09
18:2	1.7	1.7	2.6	2.39
18:3	1.5		1.4	2.06

* Number of carbon atoms: number of double bonds
** Average of winter and summer milk fat
*** Average of cis- and trans-form

Dimick and Patton (1965) used silicic acid column chromatography and low temperature crystallization from acetone of the hydrogenated milk fat to isolate the low molecular weight triglycerides from milk. The butyric acid was determined also by the use of thin-layer and gas chromatography.

II. 3. Heterogeneity and Composition of Milk Protein

II. 3. 1. Variation and Composition of Casein

Casein has long been known to consist of several components which tend to aggregate, to form the casein micelles. The exact number of casein components is unknown, but it has been estimated that there are at least twenty (Wake and Baldwin, 1961). In 1939, Mellander showed by free electrophoresis that casein contains three components. He named these components α-, β- and γ-casein in order of decreasing mobility. Other workers have confirmed these findings (Warner, 1944; Hipp et al., 1952; Von Hippel and Waugh, 1955; and MacRae and Baker, 1958).

α_s-casein Waugh and Von Hippel (1956) were the first to fractionate α-casein complex into a calcium-sensitive (α_s-casein) and calcium insensitive (k-casein) component. McMeekin et al. (1957) reported the isolation of a fraction from acid α-casein, which was

soluble at pH 4.7 and 4.0 but not soluble at pH 58-6.0. They named the fraction α_2-casein. This fraction was characterized by a low phosphorus content (1.1%) and by the fact that it could not be precipitated by calcium ions nor clotted by rennet. It was split by rennet at pH 7.3 into a soluble and an insoluble fraction. Later Hipp and his associates (1959) showed that α-casein contained a third component, α_3-casein.

Waugh et al. (1962) fractionated the calcium-sensitive fraction into two major components by the use of starch-gel electrophoresis, (6.5 M urea, pH 8.4). The component of greater mobility was termed as α_{s1}-casein and the other α_{s2}-casein. The workers attempted to fractionate the α-casein complex by use of DEAE - cellulose chromatography. The product which they obtained was termed $\alpha_{s1,2}$-casein, because it was known to contain two components. Dreizen et al. (1962) estimated, by the use of the light scattering technique, that the molecular weight of the $\alpha_{s1,2}$-casein complex was 27,300 $\pm$ 1500.

Thompson et al. (1962) showed that α_s-casein could exist in three different forms. More recently, Thompson and Kiddy (1964) renamed the α_s-casein, α_{s1}-casein, due to the possible existence of other α_s-like caseins in milk. Kiddy et al. (1964) confirmed

that the calcium sensitive components of α-casein, occurs in at least three major forms which they designated as A, B and C in order of decreasing mobility. The same workers also found that this variation is controlled by 3 allelic autosomal genes. Each allele is responsible for the production of one of the three forms of α_{s}-casein and thus six possible types and their corresponding genotypes are as follows: A(A/A), B(B/B), C(C/C), AB(A/B), AC(A/C), and BC(B/C).

Recently, Thompson and Pepper (1964) described a convenient method for the preparation of the genetic variants of α_{s1}-casein in a high degree of purity. The method involves a DEAE-cellulose-Urea (3.3M) chromatography technique.

Several workers (Manson, 1961; Waugh et al., 1962; Gordon and Basch, 1963; and Schmidt and Payens, 1963) have studied the amino acid composition of α_{s1}-casein. Kalan et al. (1964) presented evidence to show that α_{s1}-casein A, B and C have identical N-terminal residues, namely arginine, and identical C-terminal sequences namely, leucine-tryptophan.

K-casein Waugh and Von Hippel (1956) named the calcium-insensitive fraction of α-casein complex, k-casein. They reported that the k-fraction is soluble in 0.40 M calcium

chloride (pH 7.0, 0 - 4°C), forms polymer at neutral pH, and monomer at pH 12.

Several workers have published methods for the preparation of k-casein. Most of the methods make use of the solubility of k-casein in dilute calcium chloride (McKenzie and Wake, 1961; Pilson et al., 1960). Novel fractionation methods involving the solubility of k-casein in 6.6 M urea and strong acids (Zittle, 1962), 12% trichloroacetic acid (Swaisgood and Brunner, 1962) or sulfuric acid (Zittle and Custer, 1963) have been described. Rose and Marier (1963) employed column chromatography (DEAE-cellulose) for purification of k-casein.

As k-casein is involved in the stabilization of the casein micelle, it has been the subject of extensive chemical and physical investigation. Alais (1956) found that k-casein released a glycopeptide when subjected to the action of rennin or other proteolytic enzymes. The amino acids composition of the glycopeptide materials isolated from k-casein are shown in Table 11.

According to Swaisgood and Brunner (1963), k-casein consists of basic units which have molecular weight of about 60,000. They found that the molecular weight of k-casein was

reduced to 20,000 when the disulfide bonds of the protein were broken. They concluded that k-casein consists of two or more polypeptide chains which are crosslinked by disulfide.

Table 11.

AMINO ACID COMPOSITION OF k-CASEIN*(%)

Amino Acid	Analysis of		
	Jolles et al. (1962)	Swaisgood et al. (1964)	Huang et al. (1964)
Asp	7.30	7.72	8.73
Thr	6.64	6.74	6.26
Ser	6.09	5.03	5.19
Glu	17.35	19.80	19.5
Pro	8.78	10.95	11.2
Gly	1.31	1.23	1.52
Ala	5.41	5.40	4.33
Cys	1.40	---	---
Val	5.10	6.30	3.75
Met	1.00	1.68	1.16
Ileu	6.14	7.10	4.82
Leu	6.08	6.11	4.56
Tyr	7.40	7.61	6.91
Phe	4.07	3.86	3.53
Lys	5.76	6.51	0.91
His	1.67	2.36	2.20
Arg	4.00	3.96	3.31
Try	1.05	---	---
NH_3		1.94	2.20

* The values are based on a nitrogen content of 15.3%.

β-Casein Aschaffenburg (1961, 1963) first reported the existence of three variants of β-casein, (A, B, and C.) These variants could be presented either singly or in pairs in the milk of individual cows. The author showed that the synthesis of β-casein in mammary gland of the cow was under the control of three allelic genes. Later, Thompson et al. (1964) confirmed Aschaffenburg's findings that β-casein variation was breed-specific.

Thompson and Pepper (1964) compared the nitrogen and phosphorus contents of the variants of α_s- and β-casein. Table 111 summarizes their results. It is interesting to note that β-caseins contain considerably less phosphorus than do the α_s-caseins and that all the α_s-caseins contain nine residues of phosphorus.

Table 111.

NITROGEN AND PHOSPHORUS CONTENT OF α_s- AND β-CASEIN VARIANTS

Variants	Phosphorus	Nitrogen	P/N Ratio	Atoms P/mol
	(%)	(%)		
α_s-A	1.01	15.10	0.0668	9.0
α_s-B	1.01	15.34	0.0658	9.0
α_s-C	1.01	15.40	0.0655	9.0
β -A	0.59	15.18	0.0389	4.8
β -B	0.57	15.33	0.0372	4.6
β -C	0.50	15.45	0.0324	4.0

Thompson and Pepper (1964) and Gehrke et al. (1964) have developed methods for the isolation and purification of electrophoretically pure genetic variants of β-casein. The essential procedures involve chemical fractionation and subsequent purification on DEAE-cellulose column chromatography.

Gamma-casein Mellandar (1939) was the first worker to note the existence of γ-casein. He observed that the γ-casein component has a low phosphorus content. Later, Hipp et al. (1950) devised a method for the isolation of γ-casein that involves fractionation with 50% ethyl alcohol. The γ-casein prepared in this manner had an isoelectric point of pH 5.8 to 6.0 (Hipp et al. 1950). On the basis of the work of McMeekin (1954), the Protein Committee of American Dairy Science Association defined γ-casein as that the slowest-moving component in the electrophoretic pattern of skimmilk at pH 8.4-8.7, μ=0.1 (Jenness et al., 1956).

Due to the relatively small proportion (MacRae and Baker 1958) of γ-casein in the casein complex, the γ-casein is easily lost during the preparation of casein and hence may not appear in the electropherogram of whole casein (Von Hippel and Waugh, 1955).

Murthy and Whitney, (1958) reported that γ-casein was electrophoretically homogeneous in glycine hydrochloride buffer (pH 2.3 - 3.25; μ = 0.1), but was heterogeneous in a sodium lactate buffer (pH 2.3 - 3.25; μ = 0.1). The molecular weight calculated for the major components of γ-casein varied with pH and buffer ions as well as temperature [Murthy and Whitney, (1958)] and Aschaffenburg (1965) has suggested that γ-casein may exist in different polymorlic forms.

II. 3. 2. Variants of Whey Protein

<u>β-Lactoglobulin</u> β-Lactoglobulin is the principle protein component in milk whey, The prefix "β" used in the name identifies this protein with a protein which Pedersen (1936) detected in ultracentrifugal sedimentation studies on skimmilk. The name also serves to distinguish this protein from the classical "lactoglobulin" which is insoluble in half-saturated ammonium sulfate.

Aschaffenburg and Drewry (1955) examined the milk whey protein from individual cows by paper electrophoresis and found that there were two variants of β-lactoglobulin. Further study led them to conclude that the variants were the alleles of autosomal genes which occurred either singly or in pairs. The two variants were termed β-lactoglobulin A and β-lactoglobulin B

α-Lactalbumin α-lactalbumin accounts for 12% of the total whey protein (Gordon and Semmett, 1953). Recently, Robbins and Kronman (1964) described a simple method for the preparation of both β-lactoglobulin and α-lactalbumin. Aschaffenburg and Drewry (1957b) showed that α-lactalbumin might exist in three different crystal forms. Blumberg and Tombs (1958) noted the polymorphism of alpha-lactalbumin. Bhattacharya et al. (1963) confirmed the inheritance of alpha-lactalbumin in Indian Zebu cattle.

II. 4. Carbohydrate Constituents of Casein

Nitschmann et al. (1957) reported that a peptide was released from casein by rennin treatment. They found that the molecular weight of the peptide was 6,000 to 8,000, and therefore termed it a macropeptide. Alais (1956) showed that this substance was soluble in 12% trichloroacetic acid (TCA) and that it contained galactose, hexosamine and neuraminic acid.

Brunner and Thompson (1959) measured the electrophoretic mobility of the peptide in various buffers and also found that the peptide contained the following sugars: galactose

(5.1%), glucosamine (2.3%) and sialic acid (11.3%).

Malpress (1961) reported that casein which was prepared from unpasteurized skimmed milk contained, 0.21% sialic acid. Sixty eight percent of this sialic acid was released by treatment of the casein with rennin. The effects of other proteolytic enzymes (trypsin and pepsin) on casein have also been observed (Fish, 1957). However the mechanism of the proteolytic cleavage of casein has not yet been demonstrated with certainty (Beeby and Nitschmann, 1963).

Alais (1963) studied the rate of production of non-protein nitrogen (NPN) by the action of rennin on casein. He found that the rate of production of NPN ran parallel with the rate of coagulation of the casein. The NPN material was low in phosphorus but rich in sialic acid.

Johansson and Svennerholm (1956) analyzed the caseins prepared from the human milk, cow milk, goat milk, sheep milk, horse milk, reindeer milk, and whale milk for their hexose, hexosamine and sialic acid contents. Table IV shows the results of the analyses and also shows the results obtained by Baker et al. (1963) for the carbohydrate content of polar bear milk casein. Alais and Jolles (1961) have also shown that caseins

prepared from different animal milks may not have the same carbohydrate constituents.

Table IV.
Carbohydrate Content of Casein from Different Species (Weight %)

Casein	Hexose	Hexosamine	Sialic Acid
Human	1.98	1.32	0.76
Cow	0.24	0.18	0.39
Sheep	0.23	0.15	0.11
Goat	0.22	0.16	0.30
Whale	0.59	0.42	0.37
Horse	0.55	0.44	0.56
Reindeer	0.44	0.23	0.46
Polar Bear	2.80	1.09	1.92

Cayen et al. (1962) reported that alpha-, beta-, kappa- and whole casein contained 0.064 %, 0.07 %, 2.22 % and 0.38 % sialic acid respectively as compared to 0.02 %, 0.08 %. 1.28 % and 0.32 % respectively found by De Koning et al. (1963). These two groups of workers used different methods for the determination of sialic acid. It would seem, therefore, that the k-fraction contains the bulk of the sialic acid in whole casein.

Sullivan et al. (1959) used the determination of sialic acid as a special indicator for studying the distribution of k-casein in various sizes of micelles separated by differential

centrifugation. Marier et al. (1963) proposed that the k-casein content of skim milk could be estimated by determining the sialic acid content of the skim milk.

III. Material and Methods

III. 1. Collection of Milk Samples

Milk samples were obtained from five Rocky Mountain bighorn sheep living in the Jasper National Park area of Alberta, Canada. The animals were trapped in a corral-type trap furnished with gates which could be closed by remote control. The ewes were blindfolded and restrained manually while they were being milked. The milking operation was performed not later than four hours after the animals had been trapped. Although the animals were extremely excited, the milk was collected readily by the usual hand-milking procedure. The milk was strained through folded cheesecloth and was then frozen.

A sample of milk was collected from a domestic ewe (Suffolk) (Macdonald College Farm). This sample was analysed immediately after collection.

Some pertinent information concerns the five samples that were collected is presented in Table V.

III. 2. Gross Composition Analysis

III. 2. 1. Specific Gravity

Specific Gravity was determined pycnometerically, using a 2 ml. specific gravity bottle.

III. 2. 2. Total Solids and Ash

The A. O. A. C. (1960) official method was used to determine the total solids and ash of the milk samples.

Table V.
Data Pertaining to Collection of Milk Sample

Sample Number	Milk Collection Date	Months Post Partum	Age of Animal (years)
1. (Bighorn)	Sept. 4/64	3	4
2. (Bighorn)	Sept. 4/64	3	6
3. (Bighorn)			
Animal No. 1	July 17/64	1 1/2	8
Animal No. 2	July 17/64	1 1/2	3
4. (Bighorn)	July 17/64	1 1/2	9
5. (Suffolk)	Feb. 18/65	1	2

III. 2. 3. Fat Determination

Fat was determined by the Roese-Gottlieb procedure (A. O. A. C., 1960).

III. 2. 4. Lactose Determination

Lactose was determined by the method of Perry and Doan (1950).

III. 2. 5. Nitrogen Determination

Total nitrogen was determined by the micro-Kjeldahl method (A. O. A. C., 1960).

III. 3. Preparation of Methyl Ester

III. 3. 1. Preparation of Anhydrous Methanol

Anhydrous methanol was prepared by the method described by Vogel (1956). However, spectranalyzed methyl hydrate (suitable for spectrophotometry, water content 0.05%, Fisher Scientifical Company) was found to be satisfactory for fatty acid interesterification.

III. 3. 2. Preparation of Dry Hydrochloric Acid

Concentrated hydrochloric acid was led by a capillary tube from the outlet of one separatory funnel to another which contains concentrated sulfuric acid. The HCl gas was washed with sulfuric acid and was then dissolved in anhydrous methanol. The amount of hydrogen chloride in the methanol was ascertained by weighing the solution.

III. 3. 3. Fatty Acid Interesterification

The fatty acids were esterified by a modification of the method of Stoffel et al. (1959).

Milk fat (50 mg.) obtained by Roese-Gottlieb procedure and free of ether, was added to a mixture of dry benzene (0.6 ml.) and methanolic hydrogen chloride (5%, W/W; 6.0 ml.). The resultant mixture was heated under reflux (60-80° C) until a single phase was obtained (about 3 hours).

The solution was cooled to room temperature and two volumes of water were added. The resultant mixture was extracted with three, four ml. portions of petroleum ether (Redistilled, B. P. 30° C). The ether extract was neutralized and dried over a mixture of sodium sulfate and sodium bicarbon-

ate for one hour. The ether was removed from the esters by evaporation under nitrogen gas, and the esters were stored in a glass-stopped tube under nitrogen, in a vacuum desiccator.

III. 4. Preparation of Gas-liquid Chromatography Column

III. 4. 1. Equipment

The Beckman Gas-liquid chromatograph (Model GC-2) equipped with a thermal conductivity detector was employed for the fatty acid analysis. The sampling valve was removed and replaced by a straight length of 1/8 inch O. D. annealed copper tubing. This modification facilitated the direct injection of the sample material into the gas stream.

III. 4. 2. Preparation of 1, 4-Butanediol Succinate Polyester

Six gm. of 1. 4-butanediol succinate polyester (Applied Science Laboratories, State College, Pa., U. S. A.) was dissolved in 110 ml. of chloroform. Thirty-six gm. of acid-base washed Gaschrom P (60-80 mesh, Applied Science Laboratories, State College, Pa., U. S. A.) was added to the polyester solution and the mixture was allowed to stand at room temperature for 15 minutes with occasional stirring. The mixture was then placed in an oven maintained at 80° C to remove the chloroform.

III. 4. 3. Packing of Column

A straight piece of annealed copper tubing (8 ft., 1/4 in. O. D., 3/16 in. I. D.) was supported in a vertical position and loosely stoppered at one end by means of glass wool. A slight vacuum was applied to the lower end of the tube. The poly-ester-Gaschrom P mixture was added to the tube by means of a powder funnel and the tube was tapped continuously to ensure even packing. The tube was filled to within 1 cm. of the top and was then stopped with a plug of glass wool. The column was bent to form a spiral and was attached to the chromatographic unit with Swagelok fittings.

The column was conditioned by passing helium gas through it for 24 hours at a slightly higher temperature than that required in the analysis. The efficiency of the column was checked by use of a standard fatty acid mixture (Applied Science Laboratories, State College, Pa., U. S. A.).

III. 4. 4. GLC Operating Conditions

The operating conditions for analyzing the fatty acids in sheep milk fat are as follows:

Stationary phase -- 1, 4-butanediol succinate
polyester on 60-80 mesh
Gaschrom P (1:6, by weight).

Mobile phase -- Helium gas.

Flow rate -- 110 ml. per minute, measured by water displacement at the exit at room temperature and at 35 p.s.i. gauge pressure.

Column temperature-201° C. (Measured by use of a thermocouple).

Detector unit -- thermal conductivity cell.

Sample inlet temperature -- 236° C. (Measured by use of a thermocouple).

Recorder -- Bristol Dynamaster recorder at a chart speed of 1/2 inch per minute.

Sample size -- 0.5 microliter. (Hamilton microsyringe).

III. 5. Preparation of Casein and Whey

Whole casein was prepared by a modification of Warner's (1944) acid precipitation method.

Milk was centrifuged (International, Model PR-1) (50° C. 2,500 rpm) for 6 hours. The top oily liquid was removed and the middle layer was decanted from the trace of white solid which had collected at the bottom of the centrifuge tube. The middle layer,

which was a greenish-yellow milky liquid, was extracted twice with equal volumes of ethyl ether and then acidified slowly with hydrochloric acid (o. 1 N). At pH 4. 48 the solution became clear and it was assumed that the casein was completely precipitated at this point. The casein was separated from the whey by centrifugation (2, 500 rpm, 20 minutes). The slightly yellow whey was freeze-dried and stored at 5° C.

The casein precipitate was washed thoroughly with distilled water and then washed with 50%, 60%, 85% alcohol and finally with absolute ethyl alcohol. The casein was dried at room temperature and was stored in a tightly stoppered bottle.

III. 6. Starch-gel Electrophoresis

Apparatus The starch-gel electrophoresis apparatus was built in this laboratory according to the design described by Smithies (1955; 1959).

Figure IIa shows the cover plate with slot-formers. Figure IIb shows the gel tray and Figure III shows the slicing tray. The apparatus was made of Perspex glass.

Preparation of Buffer Solution Buffer No. I (Tris-citrate, pH 8. 6):- A mixture of tris (hydroxymethyl)- aminomethane ($C_4H_{11}NO_3$, purified reagent grade, Fisher Scientific Company)

(92.06 gm.) and citric acid (24 gm.) was dissolved in one liter of distilled water.

Buffer No. 2 (Borate-sodium hydroxide, pH 8.6):- A solution was prepared which was 0.3 M in respect to boric acid and 0.6 M in respect to sodium hydroxide. The reaction of the solution was pH 8.6 and was used to charge the two electrode compartments (McKinley and Read, 1961).

Preparation of Dye Solution A saturated solution of Amidoblack 10 B (Chroma-Gesellschaft and Co.) in a mixture of methanol, glacial acetic acid and distilled water (5:1:5, by volume) was prepared.

Preparation of Casein Solution Buffer No. 1 was diluted tenfold with distilled water. Sufficient urea and starch were added to the diluted buffer to adjust their concentration to 7.0 M and 2% respectively.

Procedure:- The protein preparations were analyzed by the following modification of the method of Wake and Baldwin (1961). Urea (147 gm.) was dissolved in a mixture of buffer No. 1 (35 ml.) and distilled water (200 ml.). Hydrolyzed starch (Connaught Medical Research Laboratory, University of Toronto) (52.5 gm.) were added slowly to the resultant solution followed by 2-mercaptoethanol

FIG. IIa Starch-gel Electrophoresis Coverplate and Slot-former.

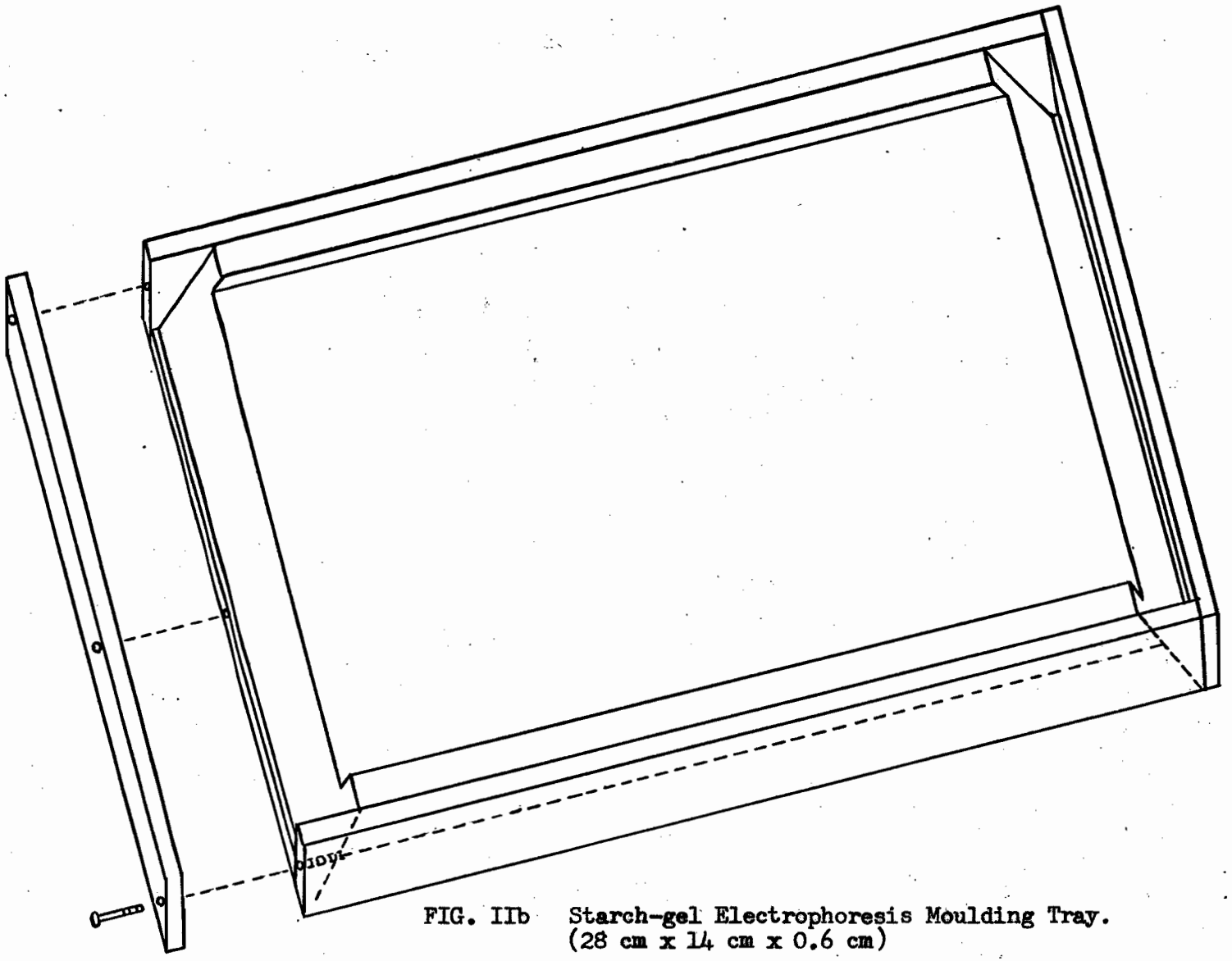

FIG. IIb Starch-gel Electrophoresis Moulding Tray.
(28 cm x 14 cm x 0.6 cm)

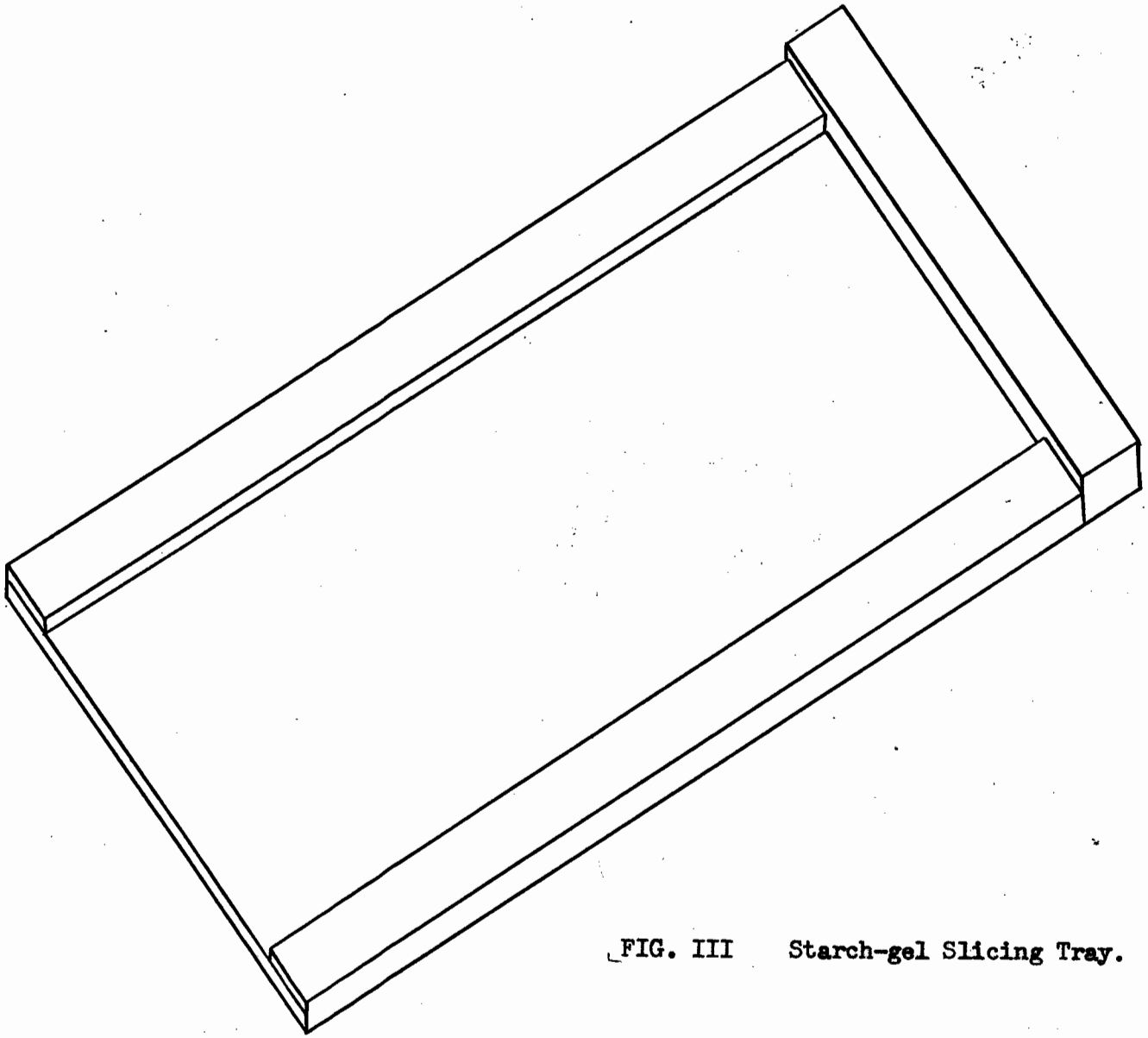

FIG. III Starch-gel Slicing Tray.

(Eastman Organic Chemicals) (0.7 ml.). The final volume of the mixture was 350 ml. The mixture which was contained in an air-tight stoppered Erlenmeyer flask (500 ml.) was heated in a water bath (80° - 95° C) until the solution became clear. The flask was swirled continuously and gently during the heating operation.

The gel solution was poured carefully into a starch-gel electrophoresis tray (Figure IIb) in order to avoid the trapping of air bubbles. Cover plate (Figure IIa) was placed in position and lead weights were pressed on the cover at the corners. The gel was allowed to stand at room temperature for three hours. The slot-formers were then removed carefully and the casein solutions were placed in the slots by means of droppers. Each slot was filled completely. Liquified Petrolatum N. F. (Fisher Scientific Company) was poured over the gel to seal the sample slots and to avoid the loss of moisture from the gel. Vertical electrophoresis was performed by use of the assembly shown in Figure IV in cold room (5° C). A pontential gradient of 250 volts was applied across the system for the period of 16 to 20 hours.

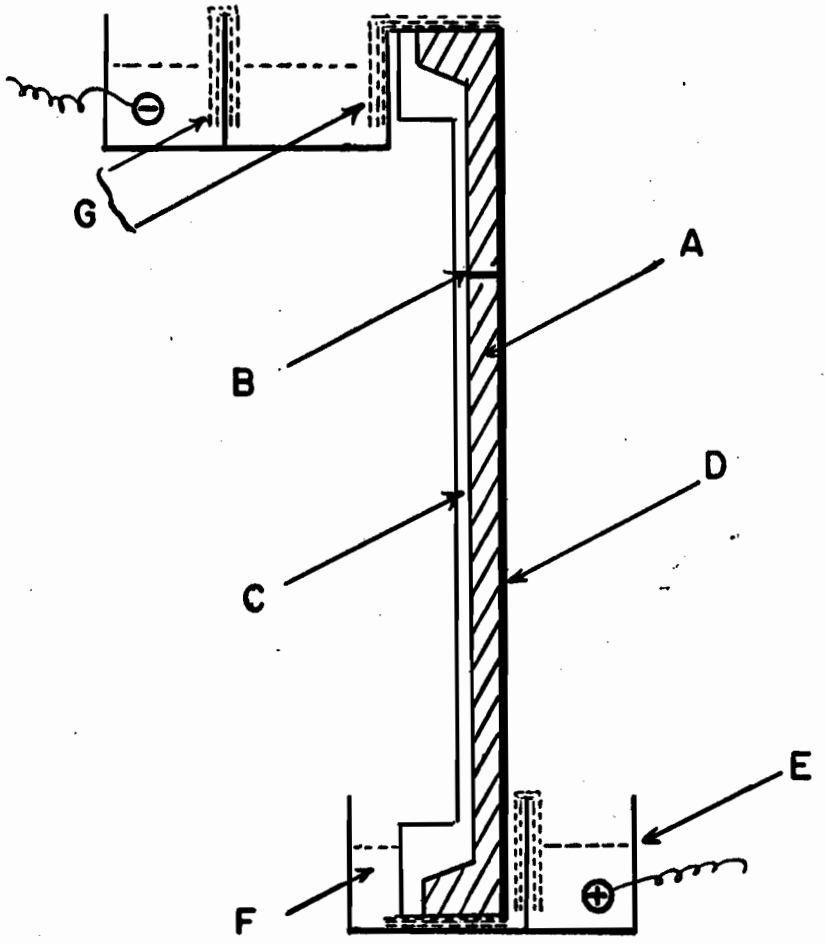

FIG. IV Diagram of Vertical Starch-gel Electrophoresis.
A = Starch gel; B = Sample slots;
C = Starch-gel tray; D = Petrolatum seal;
E = Electrode compartment;
F = Bridge solution; G = Filter-paper bridge.

At the end of the electrophoresis, the Petrolatum was removed and the gel plate was transferred to the slicing tray (Figure III) where it was sliced longitudinally by means of a Blair Brown knife blade. The bottom half of the starch plate (0.3 cm. thickness) was stained for 5 minutes with saturated Amidoblack 10 B solution. After the staining operation the gels were thoroughly washed with the solvent mixture to remove the excess dye. The gels were wrapped in Saran Wrap and stored at 5° C.

III. 7. Polyacrylamide-gel Electrophoresis

Apparatus A cell for horizontal polyacrylamide-gel electrophoresis was constructed of Perspex glass. A diagram of the apparatus is shown in Figure V. The electrical current was supplied by a Spinco Duostat (Serial No. 436, Specialized Instruments Corp., California, U.S.A.).

Preparation of Buffer Solution Buffer No. 1 (Tris-EDTA-Borate, pH 9.1-9.3):- Tris(hydromethyl)-amino-methane (121 gm.) disodium ethylenediamine tetraacetate (15.6 gm.) and boric acid (9.2 gm.) were dissolved in four liters of distilled water. One part of this stock solution was diluted with two parts

of distilled water to give a solution of pH 9.1-9.3. This buffer was used for the preparation of the polyacrylamide gel for casein analysis.

Buffer No. 2. (Tris buffer, pH 9.1-9.4):- A tris (hydroxymethyl)aminomethane solution (0.02 M, 220 ml.) was diluted with distilled water (1250 ml.) to give a solution of pH 9.1-9.4. This buffer was used for preparation of the polyacrylamide gels for whey protein analysis.

Buffer No. 3 (Borate-sodium hydroxide buffer, pH 8.6):- This was the same buffer as buffer No. 2 which was used in the previous section.

Buffer No. 4. (Borate-lithium hydroxide buffer, pH 8.6):- Solid lithium hydroxide was added to a boric acid solution (0.3 M) until the reaction of the mixture was pH 8.6.

Preparation of Dye Solution Amidoblack 10 B (1 gm.) was dissolved in methanol (250 ml.) and the resultant solution was added to a mixture of glacial acetic acid (100 ml.) and distilled water (650 ml.). The solution was then filtrated to remove insoluble material (Aschaffenburg, 1964).

Preparation of Gel Solution Gel Solution No. 1:- Cyanogum (Cyanogum 41, gelling agent, Fisher Scientific Company) (70 gm.),

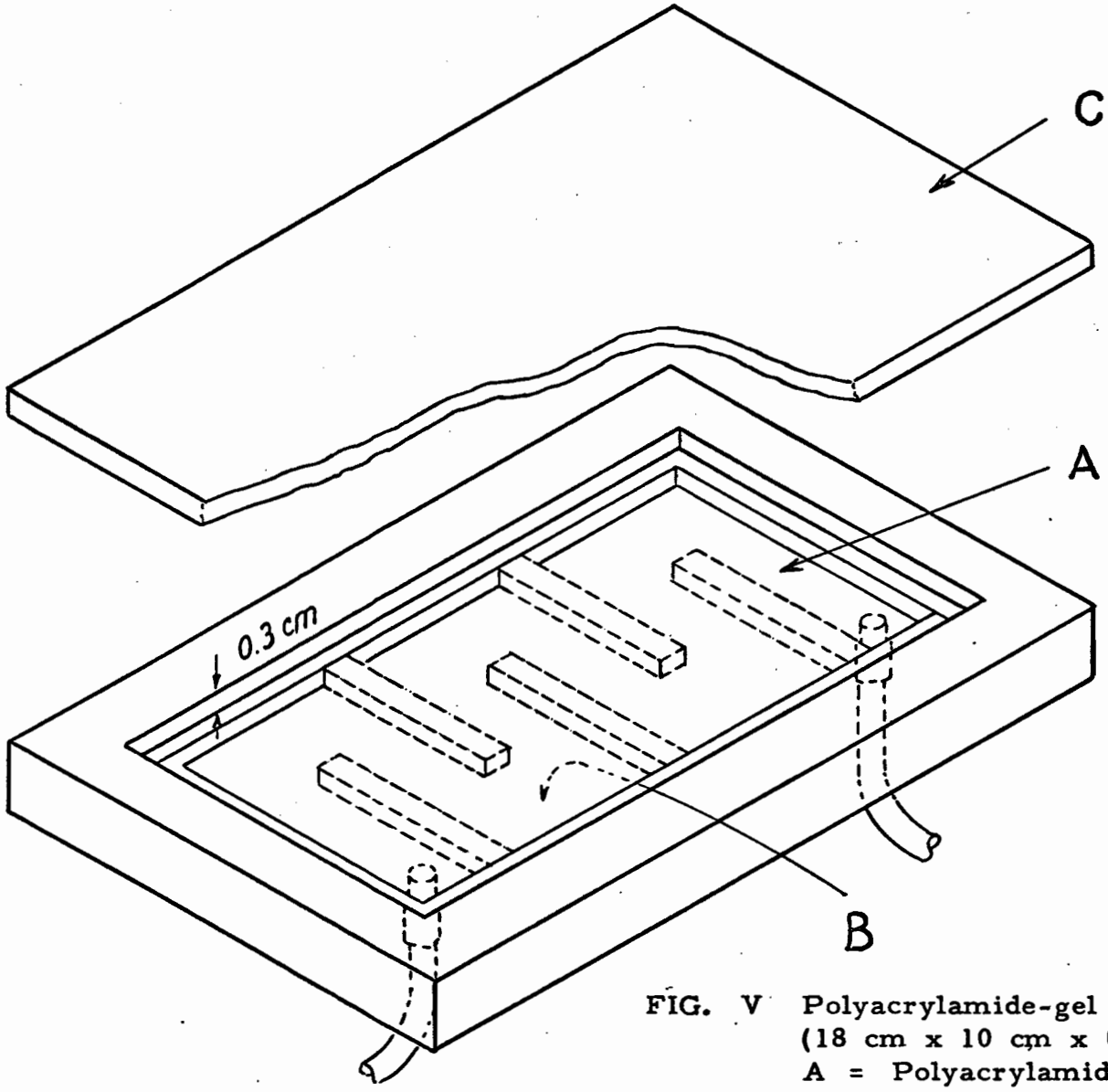

FIG. V Polyacrylamide-gel Electrophoresis Tray.
(18 cm x 10 cm x 0.3 cm)
A = Polyacrylamide gel,
B = Water-cooling chamber,
C = Coverplate.

urea (270 gm.) and 3-dimethylamino-propionitrile (Eastman Organic Chemicals) (1 ml.) were dissolved in buffer solution No. 1 and the volume was adjusted to one liter. The solution was then filtrated and stored in an air-tight bottle. This solution was suitable for casein analysis and could be stored at room temperature for several weeks.

Gel Solution No. 2:- Cyanogum (70 gm.) was dissolved in buffer No. 2 and made up to one liter. The solution was suitable for whey protein analysis and was stable for several weeks at room temperature.

Procedure The casein preparations were analyzed by a modification of the method of Thompson et al. (1964) and the whey preparations were analyzed by a modification of the method of Aschaffenburg (1964).

The procedures which were followed for casein and whey analyses by means of polyacrylamide-gel electrophoresis were the same, except that the urea-free buffers were used for whey analysis.

The desirable gel solution (80 ml.) and freshly prepared ammonium persulfate solution (0.2 gm. / ml. of buffer No. 1 or No. 2) (1 ml.) were placed in a 150 ml. flask and the flask was

swirled for 1 minute. The gel was then carefully poured into the gel-moulding tray which was then covered with the Perspex cover. Atmospheric oxygen was excluded and seepage of the gel solution was prevented by greasing the margins of the tray with Petrolatum N. F. The gel was allowed to stand for forty-five minutes and the cover plate was then lifted.

The solid gel was cut transversely about 4 cm. from its cathodic end by means of a thin blade scalpel. Strips (up to five) of Whatman No. 3 MM filter paper (1.2 x 0.25 cm.) which were soaked in the test solution (1% casein in buffer No. 1 or whey in buffer No. 2) and freed from excess solution by placing the strips on soft tissue paper, were inserted into the slits by use of a stainless steel forceps. Care was taken to avoid the trapping of air in the slit.

The gel was then connected to the electrode solution (buffer No. 3 or No. 4) by means of several layers of Whatman No. 3 MM filter paper. The gel tray and the electrode vessels were covered with a piece of Saran Wrap to avoid the loss of moisture and cold tap water was circulated through the apparatus. A diagram of the assembly is shown in Figure VI. The voltage was first set at 150 V. and then increased to 250 V. after 15 minutes. The voltage was maintained at 250 V. for three hours. The current was about 50 mA at the beginning of the run but then dropped to about 30 mA at the end of the experiment.

POLYACRYLAMIDE-GEL ELECTROPHORESIS

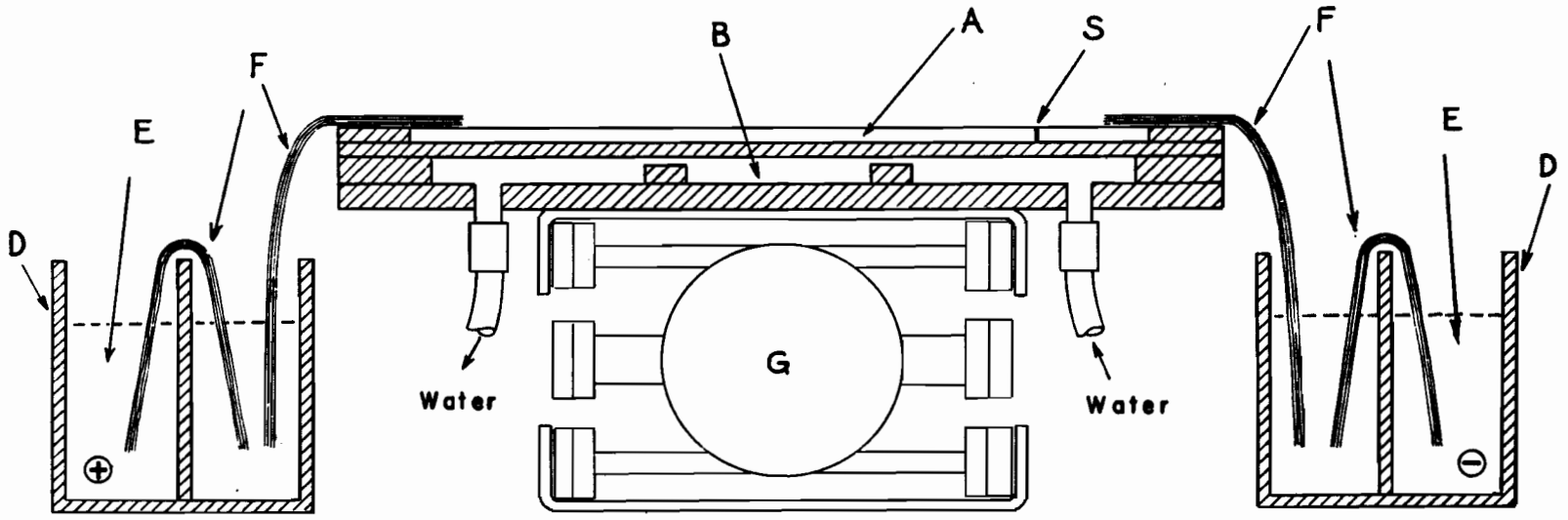

FIG. VI Diagram of Horizontal Polyacrylamide-gel Electrophoresis.
A = Polyacrylamide gel, B = Circulating cold water,
S = Samples (absorbed in filter paper strips),
D = Electrode comparments, E = Bridge buffer,
F = Filter paper wick, G = Lab jack.

The gel was removed from the tray and was allowed to stand in the dye solution for three minutes. The gel was then washed thoroughly with acetic acid (10%, V/V). Gels were wrapped in Saran Wrap and stored at 5° C.

III. 8. Determination of Sialic Acid in Sheep Casein

Sialic acid was determined by the thiobarbituric acid method of Warren (1959). Synthetic N-acetylneuraminic acid (prepared by Huang and Baker, 1964) was used for the preparation of the calibration curve.

Hydrolysis of Casein A mixture of casein (15 mg.) and sulfuric acid (2 ml., 0.05 N) was heated (95° C) in a sealed tube for 30 minutes (Huang and Baker, 1964). The tube was cooled and opened. The hydrolysate was then centrifuged (1500 rpm, 2 hours) to remove the insoluble material and was then evaporated to dryness in a vacuum desiccator. The residue was dissolved in distilled water (1 ml.) and the resultant solution was analyzed for sialic acid content.

III. 9. Determination of Hexose and Hexosamine in Sheep Casein

The hexose content of the sheep milk casein was determined

by the method of Montgomery (1961), and hexosamine by the method of Anastassiadis and Common (1955, 1958). D(+)-Galactose and glucosamine-hydrogen chloride were used for the preparation of the standard curves.

Hydrolysis of Casein A mixture of casein (20 mg.) and resin suspension (5 ml.) (one part of Dowex 50 x 8, 200-400 mesh, in four parts of 0.02 N hydrochloric acid) was heated (rotary oven, 105° C) for 24 hours. The tubes were cooled, opened and the contents were placed in a sintered-glass chromatographic tube (350 x 12 mm.). The eluate was collected and the resin column was then washed with 10 ml. distilled water. The column was then eluted with hydrochloric acid (10 ml., 2 N). The acid eluate was evaporated to dryness to remove excess hydrochloric acid and the residue was dissolved in water. Hexosamine was determined on the resultant aqueous solution.

IV. Results and Discussion

IV. 1. Gross Composition of Bighorn Sheep Milk

The composition of milk may vary considerably. Some of the factors which affect the composition of milk are as follows:- breed, age, stage of lactation, diet, individual genetic factors and environment. (Macy et al., 1953; Jenness and Patton, 1959; Kon and Cowie, 1961). For example, the total solids tends to decrease with successive lactation periods (Parrish et al., 1948); the colostrum contains more mineral salts, protein and less lactose than does normal milk (Parrish et al., 1950) and the ash, protein and fat contents are higher during the summer months (Overman, et al., 1939).

Table VI shows the gross composition, specific gravity and pH of the various milk samples. Each value shown in the Table represents the average of three actual determinations.

The total solids of the milk collected at three months post partum (sample No. 1 and No. 2) were considerably higher than those of the milk collected at one and one half months post partum (sample No. 3 and No. 4). It was observed that the milk samples which had the higher solids content were much more viscous than those which had the lower solids content.

Table VI
Gross Composition of Sheep Milk*

Determination	Sample No.				
	1	2	3	4	5**
Total Solids (%)	32.6	35.3	19.5	18.6	14.3
Water (by difference) (%)	67.4	64.7	80.5	81.4	85.7
Ash (%)	1.34	1.28	1.18	0.93	0.87
Fat (%)	15.8	16.1	8.50	7.77	4.01
Solids-not-fat (by difference) (%)	16.7	19.2	11.0	10.8	10.3
Lactose (%)	2.68	4.13	5.02	5.33	5.15
Total Protein (%) (N x 6.38)	13.6	10.4	5.13	6.03	4.29
Specific Gravity (23° C)	1.0448	1.0156	1.0118	1.0128	1.0370
pH value	6.31	6.45	6.37	6.33	6.70

* Average of three determinations.
** Domestic (Suffolk) sheep milk.

The milk samples which were collected during the summer months and were obtained from older animals (sample No. 3 and No. 4) contained less fat and protein and more lactose than did the other samples.

Bonnier et al. (1946) reported that the fat and the protein contents of bovine milk increased as the end of lactation period approached. Bailey (1952) reported that this increase is less pronounced with older cows. Recently, Nejim (1963) analyzed the milk of Iraq Awassi sheep over a whole lactation period (about five months). The results he obtained were as follows:- specific gravity 1.0305-1.0415; pH 6.46-6.90; fat 5.7-11.8%; protein 5.85-6.50%; lactose 4.65-7.04%; total solids 17.34-24.56%; solids-not-fat 11.11-13.92%; ash 0.864-1.018%. It will be noted that the ranges of the results are not as great as those obtained from bighorn sheep milk.

Blood (1965, personal communication) reported that the mean date for parturition of Rocky Mountain bighorn sheep in the Jasper Park area is June 1st, and that the mother suckles her offspring for a period of three to five months. It could be, therefore, that sample No. 1 and sample No. 2 were collected from animals at the very end of the lactation period.

One might conclude either that the composition of bighorn sheep milk is affected greatly by stage of lactation, age, seasonal temperature, or that the milk from individual animals differs markedly in composition.

A comparison of the composition of bighorn sheep with that of domestic sheep shows that the former contains a considerably higher percentage of fat and protein than the latter. This could be due to the circumstance that the bighorn sheep lambs need a highly nutritious diet to cope with the more rigorous environment and to promote an early rapid growth of the young.

Table VII shows the gross composition of sheep milk obtained from several different species. It will be noted that bighorn sheep milk collected at one and one half months post partum is similar in protein and fat content to the milk of Egyptian, Polish, Bulgarian and Czechoslovakian sheep. The domestic (Suffolk) sheep milk is similar in composition to Sardinian and New Zealand Clun Forest sheep milk.

Table VII
Comparison of Gross Composition of Several
Different Sheep Milks (Weight Percentage)

Determination	Analysis of					
	El-Sokkary et al. (1949)	Jankowski (1953)	Iotov and Ikonomov (1957)	Prekapp (1957)	Sartore and Lai (1957)	Ashton et al. (1964)
	Egyptian sheep	Polish sheep	Bulgarian Tsigai sheep	Czechoslo-vakian sheep	Sardinian sheep	New Zealand sheep
Total Solids	19.35	18.99	18.67	19.4	15.71-17.43	16.9
Ash	---	0.86	0.82	0.9	-----	0.87
Fat	8.0	8.45	7.2	7.7	6.25- 7.87	6.2
Solid-not-fat (by difference)	11.35	10.54	11.47	11.7	11.48-12.42	10.7
Lactose	---	4.91	4.31	4.5	4.23- 5.01	4.7
Total Protein	5.87	5.24	6.07	6.3	5.02- 8.00	5.3
Specific Gravity	1.035	1.0347	---	1.037	1.0373-1.0395	1.035

IV. 2. Fatty Acid Composition of Bighorn Sheep Milk

The various samples of sheep milk fat were analyzed for fatty acid constitution by gas-liquid chromatography. Figure VIIa, VIIb and VIIc show typical chromatograms which were obtained. It will be noted that twenty-three components, including three isomeric forms, were detected in all five samples.

Table VIII shows the results of the quantitative estimation of fatty acids in sheep milk fat. Each figure represents the average of two determinations. All five samples contain fairly high amounts of lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid.

Bartley *et al.* (1951) reported that the stage of lactation influences to a marked degree the unsaturated fatty acid content of butter fat. For example, they showed that the oleic acid and the linoleic acid content of bovine fat was high at the beginning of the lactation period and decreased as the end of the lactation period approached. Hilditch (1956) noted that the butyric acid content of sheep milk fat was somewhat lower than it was in bovine milk fat. The author's findings appear to be in agreement with both the works of Bartley and Hilditch.

FIG. VIIa Gas-liquid Chromatograms of Fatty Acids in Bighorn Sheep Milk Fat, Sample No. 1 & 2.

Peak number and corresponding fatty acid:

1 = 4:0	2 = 6:0	3 = 8:0
4 = 9:0	5 = 10:0	6 = 10:1
7 = 11:0	8 = 12:0	9 = 13:0
10 = 14:0 iso	11 = 14:0	12 = 14:1
13 = 15:0	14 = 16:0 iso	15 = 16:0
16 = 16:1	17 = 17:0 iso	18 = 17:0
19 = 17:1	20 = 18:0	21 = 18:1
22 = 18:2	23 = 18:3	

FIG. VIIa Gas-liquid Chromatograms of Fatty Acids in Bighorn Sheep Milk Fat, Sample No. 1&2.

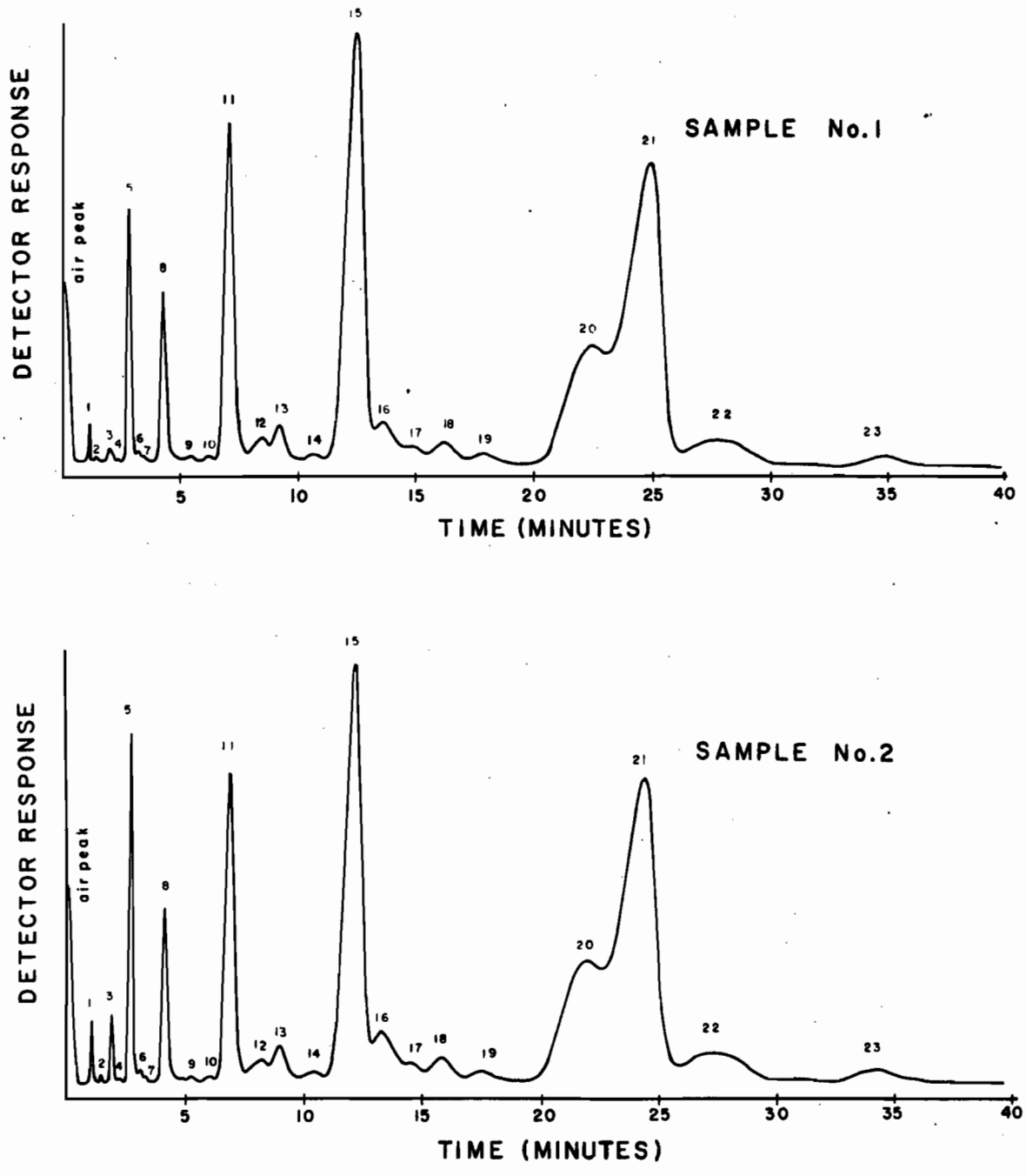

FIG. VIIb Gas-liquid Chromatograms of Fatty Acids in Bighorn Sheep Milk Fat, Sample No. 3 & 4.

Peak number and corresponding fatty acid:

1 = 4:0	2 = 6:0	3 = 8:0
4 = 9:0	5 = 10:0	6 = 10:1
7 = 11:0	8 = 12:0	9 = 13:0
10 = 14:0 iso	11 = 14:0	12 = 14:1
13 = 15:0	14 = 16:0 iso	15 = 16:0
16 = 16:1	17 = 17:0 iso	18 = 17:0
19 = 17:1	20 = 18:0	21 = 18:1
22 = 18:2	23 = 18:3	

FIG. VIIb Gas-liquid Chromatograms of Fatty Acids in Bighorn Sheep Milk Fat, Sample No. 3&4.

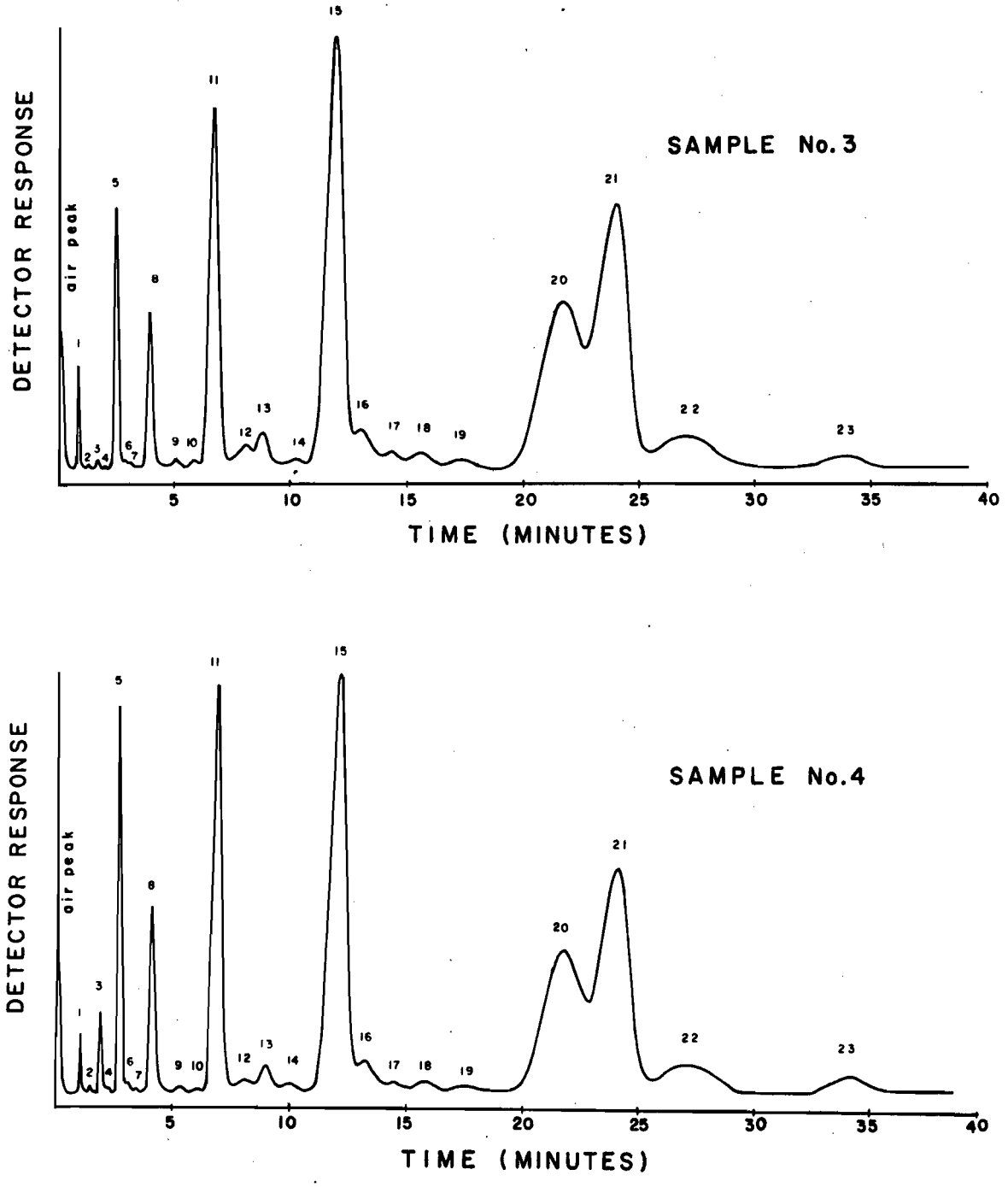

FIG. VIIc Gas-liquid Chromatograms of Fatty Acids in Domestic (Suffolk) Sheep Milk Fat.

Peak number and corresponding fatty acid:

1 = 4:0	2 = 6:0	3 = 8:0
4 = 9:0	5 = 10:0	6 = 10:1
7 = 11:0	8 = 12:0	9 = 13:0
10 = 14:0 iso	11 = 14:0	12 = 14:1
13 = 15:0	14 = 16:0 iso	15 = 16:0
16 = 16:1	17 = 17:0 iso	18 = 17:0
19 = 17:1	20 = 18:0	21 = 18:1
22 = 18:2	23 = 18:3	

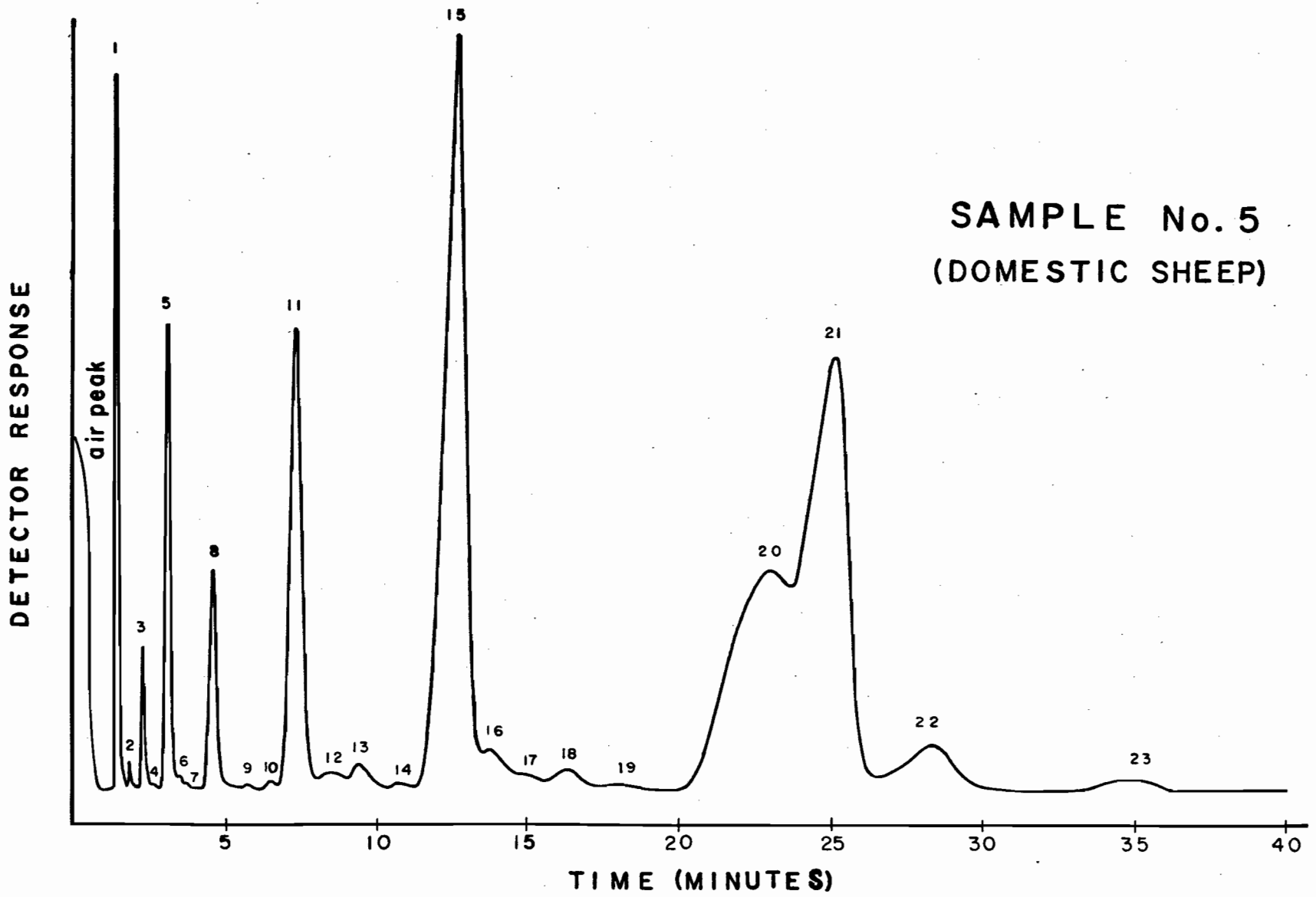

FIG. VIIc Gas-liquid Chromatogram of Fatty Acids in Domestic (Suffolk) Sheep Milk Fat.

Table VIII
Fatty Acid Composition of the Ether-soluble
(Roese-Gottlieb) Portion of Sheep Milk
(Weight % of Total Eluate)

Components	Sample No.				
	1	2	3	4	5
4:0*	0.25	0.35	1.21	0.38	2.47
6:0	T**	T	T	T	T
8:0	0.14	0.50	T	0.50	0.97
9:0	T	T	T	T	T
10:0	2.98	3.86	3.15	4.58	2.97
10:1	0.15	0.17	T	0.10	0.11
11:0	T	0.11	T	T	0.13
12:0	2.66	2.70	2.19	2.94	2.27
13:0	0.12	0.14	0.16	T	0.19
14:0 iso	0.13	0.12	0.20	T	T
14:0	9.58	8.08	9.37	12.23	7.81
14:1	1.09	1.10	1.29	0.80	0.39
15:0	1.26	1.31	1.18	1.09	0.70
16:0 iso	0.49	0.41	0.40	0.43	0.29
16:0	21.99	19.87	20.58	22.91	2 2.02
16:1	2.72	3.39	2.47	2.19	1.57
17:0 iso	0.84	0.78	0.85	0.67	0.39
17:0	1.65	2.27	1.65	0.72	2.10
17:1	0.58	0.80	0.48	0.34	1.05
18:0	16.89	17.43	21.53	19.05	1 8.35
18:1	31.18	30.49	25.18	23.89	3 0.43
18:2	4.27	4.47	5.27	4.90	4.72
18:3	0.94	1.60	2.50	2.05	0.78

* Number of Carbon : Number of double bonds.
** T indicates trace (smaller than 0.1%).

The results reported in the present study suggest (a) that the fat from bighorn sheep milk may contain a smaller proportion of short-chain fatty acids than does the fat from domestic sheep milk, and (b) that sheep milk in general may contain a higher proportion of stearic and linoleic acid than does bovine milk (see Table I).

IV. 3. Electrophoretic Analyses of Sheep Casein and Whey

Figure VIII shows typical starch-gel electrophoretic patterns of sheep milk casein. It will be noted that each of the five samples of casein contained two major components designated as α- and β- casein in order of decreasing mobility. The four samples of bighorn sheep gave k-casein bands but the domestic sheep casein appeared to contain no k-casein. Many small bands were observed on the casein electropherograms, some of which do not appear in the photograph. One might conclude either that the k-casein content of domestic sheep casein is less than that of bighorn sheep casein or that the k-casein of domestic sheep casein complex was not resolved under the conditions of the experiment.

Sample No. 1 and No. 4 have a pre-α s zone. It is possible that polymorphism and genetic variation exist in big-

horn sheep casein as in bovine milk casein. However, due to the limited number of bighorn sheep milk samples, a conclusive genetic typing of all possible variants has not been attempted in the present study.

Figure IX shows polyacrylamide-gel electrophoretic patterns of sheep caseins. Each casein sample gave α_s- and β-casein zones and each of the α_s- and β-caseins of bighorn sheep milk contains two components. It is interesting to note that the mobilities of the corresponding components of the sheep caseins were similar and that the mobility of the corresponding components of cow casein were higher than those of the sheep milk components.

The electropherograms which are represented in Figure IX were scanned on a densitometer (Densicord, Model 542, Photovolt Corporation, N. Y.) and the resultant absorption curves are represented in Figure X. The curves show that each of the α_s- and β-zones comprise more than one component. No attempt was made to estimate quantitatively the relative amount of each fraction, because (a) light absorption does not obey Beer's Law at high protein concentrations, (b) the dye binding capacities of the protein components may be different and (c) the peak in each of the α- and β- zones are so close together that an accurate estimation of the individual components is not feasible.

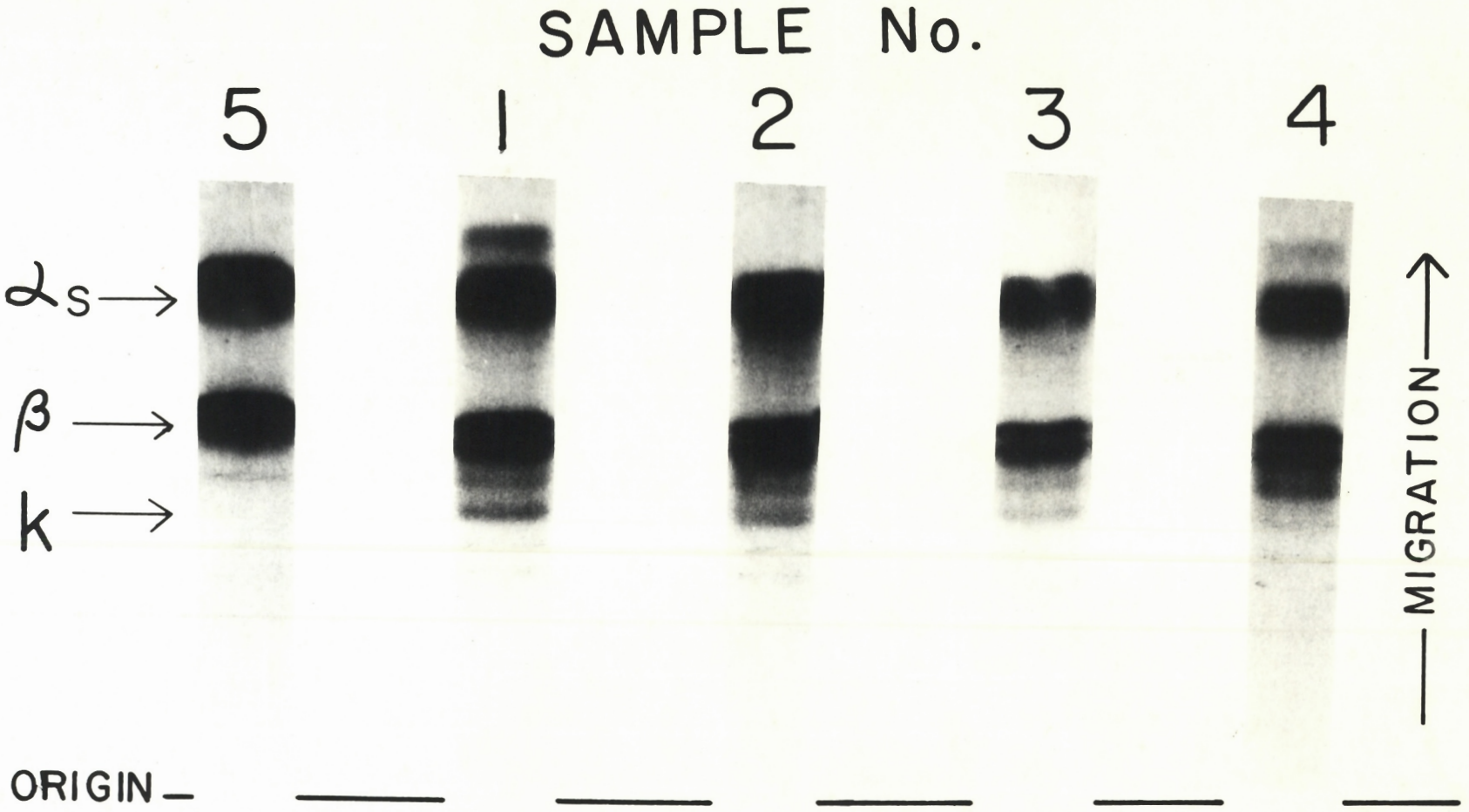

FIG. VIII Starch-gel electrophoresis patters of sheep milk casein. 1-4: Bighorn sheep casein; 5: Domestic (Suffolk) sheep casein.

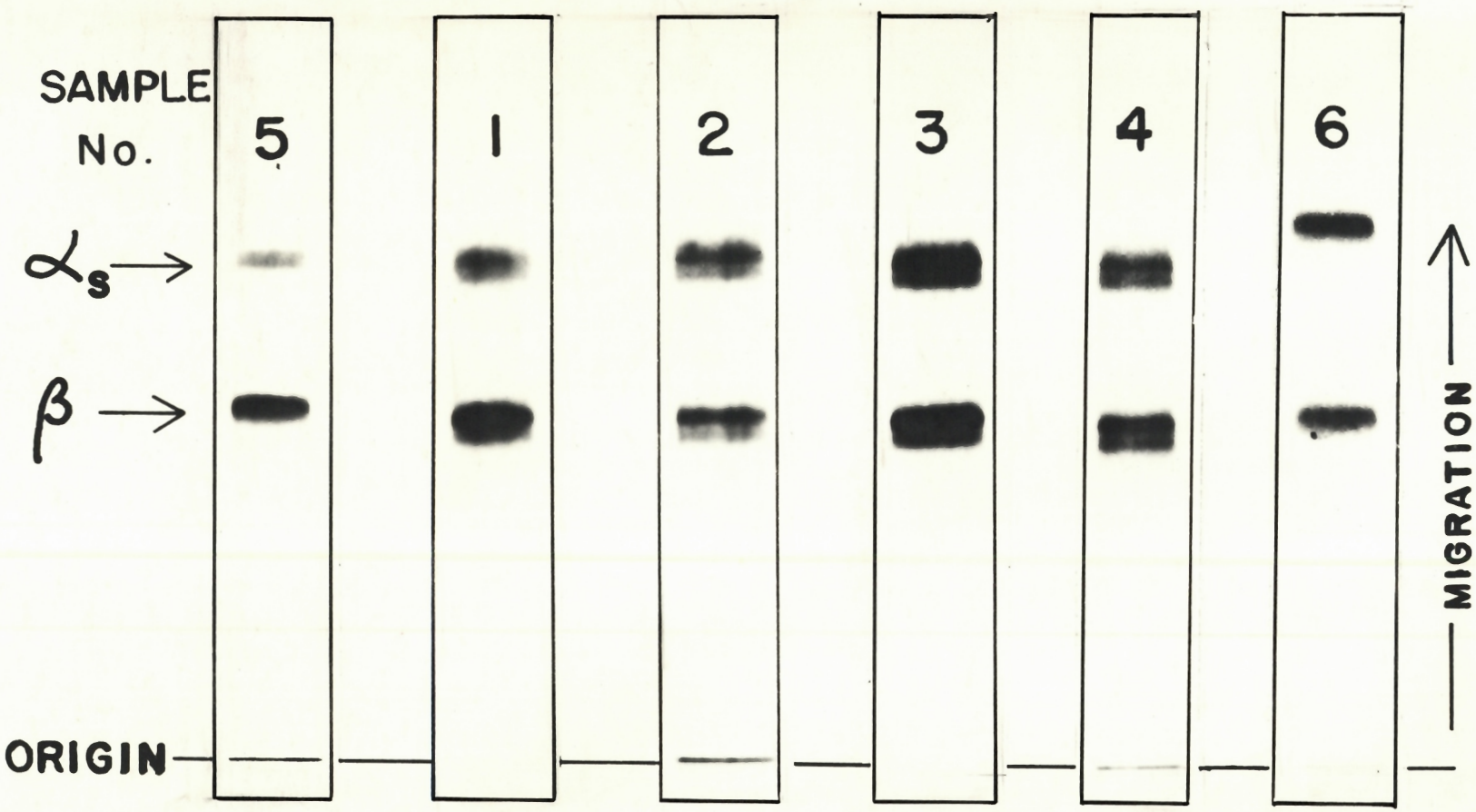

FIG. IX Polyacrylamide-gel electrophoresis patterns of casein. 1-4: Bighorn sheep; 5: Domestic sheep casein; 6: Cow casein.

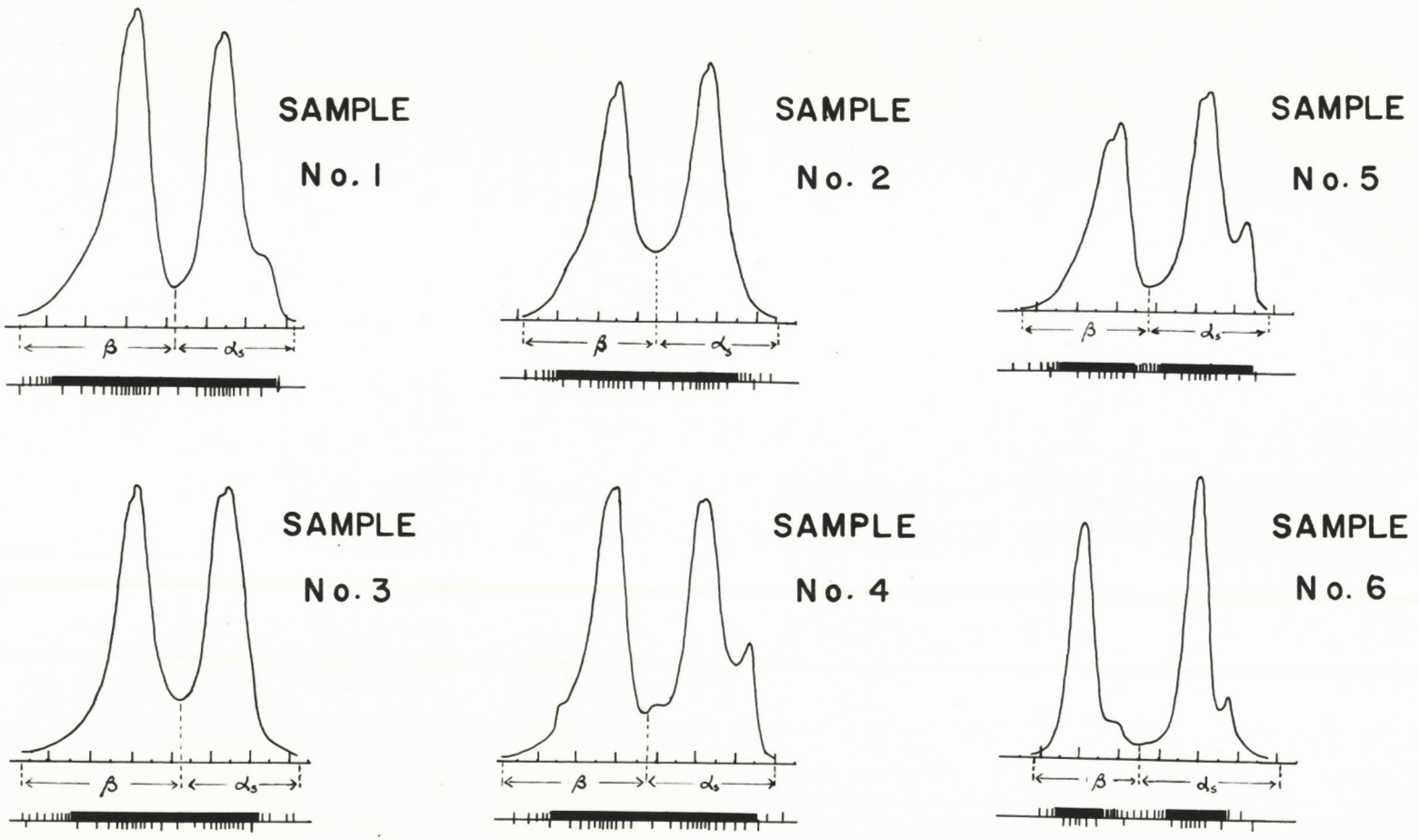

FIG. X ABSORPTION CURVES OF THE POLY-ACRYLAMIDE-GEL ELECTROPHEROGRAMS SHOWN IN FIG. IX

Sloan et al. (1961) studied the paper electrophoretic behaviour of caseins prepared from the milk of mammals of the Ovis genus and obtained similar results to those reported in the present study.

The whey which was obtained from the preparation of bighorn sheep milk caseins and domestic sheep milk casein was freeze dried. Appropriate solutions of the freeze dried whey samples were analyzed by polyacrylamide-gel electrophoresis. A sample of bovine whole milk which was known to contain β-lactoglobulin A/B*, was analyzed at the same time for the purpose of comparison. Figure XI shows the results of these analyses. Electropherogram A illustrates the position of β-lactoglobulin A/B of bovine milk. Electropherograms 1 to 5 illustrate that the β-lactoglobulin fraction of bighorn sheep milk and domestic sheep milk contains two electrophoretic components. Electropherogram 4+B was prepared using a solution of bighorn sheep milk casein and freeze dried whey. A sufficient quantity of freeze dried whey was employed in order to show the position of the β-lactoglobulin fraction in relation to those of α- and β- caseins. It will be noted that the β-lactoglobulin fraction runs ahead of the α-casein fraction.

* This milk was supplied by Dr. H.F. McRae, Department of Animal Science, and had been established as type A/B by polyacrylamide gel electrophoresis.

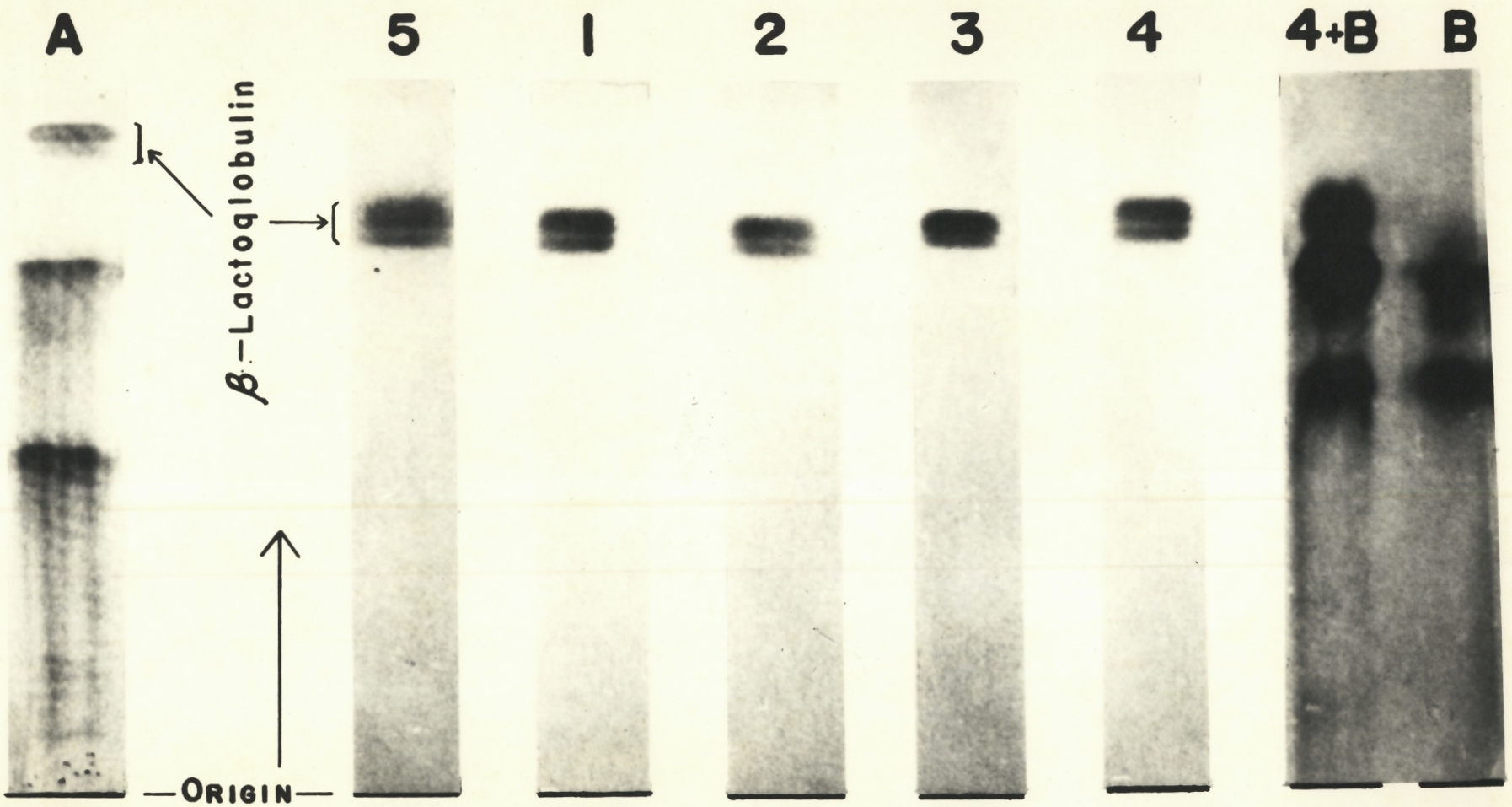

FIG. XI Polyacrylamide-gel Electropherograms of Sheep Milk Whey. A: Bovine Whole Milk; B: Bighorn Sheep Milk Casein; 1-4: Bighorn Sheep Whey; 5: Domestic (Suffolk) Sheep Whey.

IV. 4. Carbohydrate Content of Bighorn Sheep Casein

Figure XII shows the calibration curves which were obtained by the use of galactose, glucosamine-hydrogen chloride and N-acetylneuraminic acid. Table IX lists the hexose, hexosamine and sialic acid contents of the various sheep milk caseins. Each value represents the average of four independent determinations.

It is interesting to note that the caseins prepared from the various samples of bighorn sheep milk contain a higher percentage of hexose, hexosamine and sialic acid than does the domestic sheep casein.

Table X shows a comparison of the carbohydrate contents of caseins prepared from bighorn sheep milk, domestic sheep milk, cow milk and goat milk. It will be noted that caseins prepared from bighorn sheep milk contains more hexose than does that prepared from domestic sheep milk, cow milk and goat milk. However, the bighorn sheep milk casein appears to contain less sialic acid than do the other casein samples.

The values for the sialic acid content of domestic sheep milk which were obtained in the present study were somewhat lower than those reported by Johansson and Svennerholm (1956). This could be due to the higher specificity of the Warren method as compared to

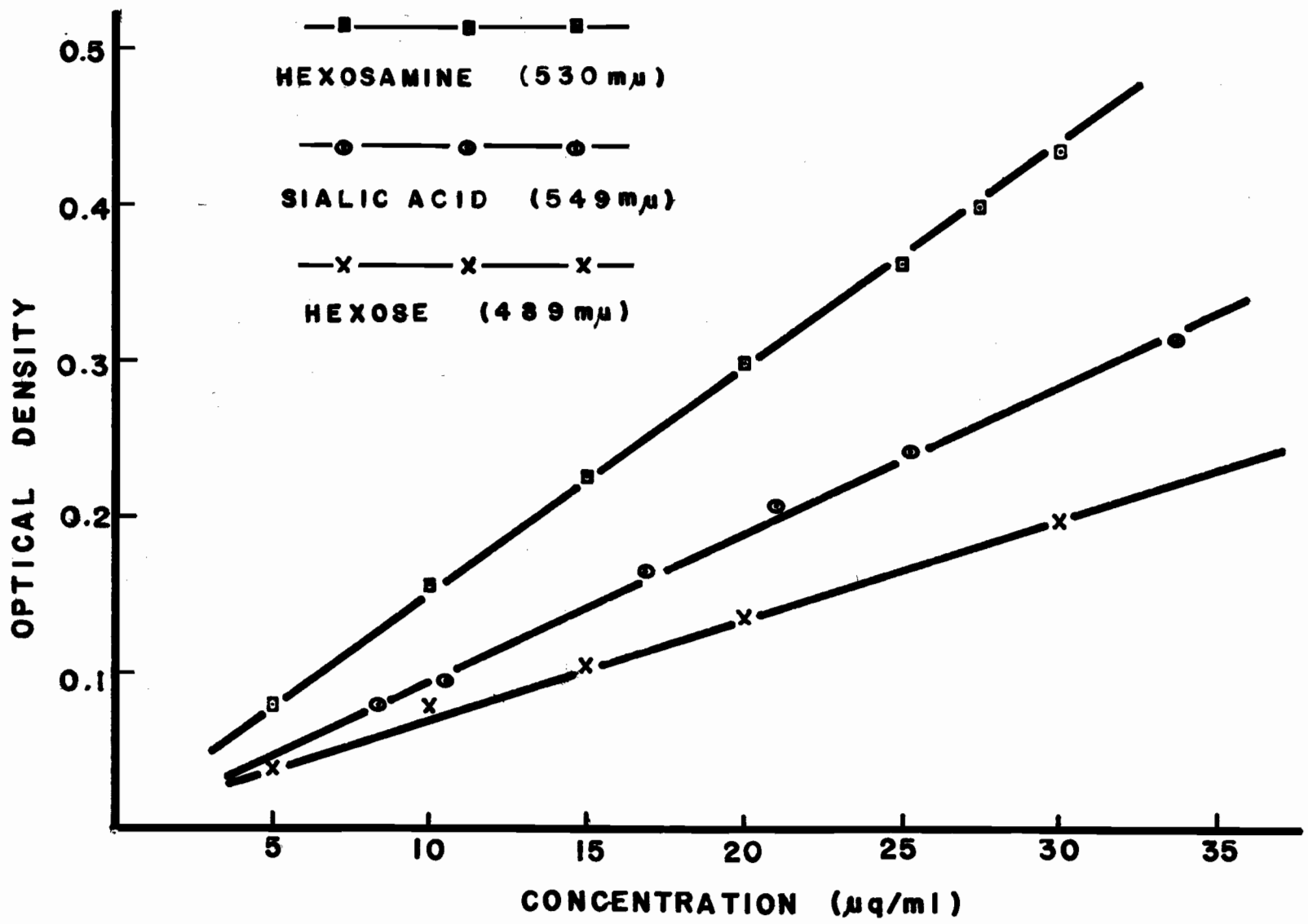

FIG. XII Calibration Curves for the Determination of Carbohydrates in Sheep Casein.

Table IX

Carbohydrate Content of Sheep Casein*

(mg. / g.)

Determination	Sample No.				
	1	2	3	4	5**
Hexose	3.38	4.47	4.01	4.30	2.37
Hexosamine	1.37	2.08	1.29	1.68	1.02
Sialic Acid	1.50	1.73	1.42	1.37	0.98
Total Carbohydrate	6.25	8.28	6.72	7.35	4.37

* Average of four independent determinations

** Domestic (Suffolk) sheep casein

the Svennerholm (1957) method. Baker et al. (1963) and DeKoning et al. (1963) made similar observations in connection with the sialic acid content of bovine casein.

Table X.
Comparison of Carbohydrate Content of
Bighorn Sheep Casein with Sheep,
Cow and Goat Casein.
(Weight %)

Casein	Hexose	Hexosamine	Sialic Acid
Bighorn Sheep	0.40*	0.16*	0.15**
Sheep**	0.23	0.15	0.11
Cow**	0.24	0.18	0.39
Goat**	0.22	0.16	0.36

* Average value for the four samples of bighorn sheep milk caseins.

** Results obtained by Johansson and Svennerholm (1956).

(1) Milk was collected from five Rocky Mountain bighorn sheep living in the Jasper National Park of Alberta. Milk was also collected from a domestic (Suffolk) sheep.

(2) The gross composition of the milk sample was determined by conventional method. The range of values which were obtained for each determination are as follows: total solids, 18.6-32.6%; ash, 0.93-1.34%; fat, 7.77-16.1%; lactose, 2.68-5.33%; protein, 5.13-13.6%. The domestic sheep milk contained considerably lower fat and protein than did the bighorn sheep milk.

(3) The fatty acid constitution of the sheep milk fat was estimated by gas-liquid chromatography. The average amounts of the major fatty acids in the bighorn sheep milk fat were as follows: lauric, 2.62%; myristic, 9.81%; palmitic, 21.34%; stearic, 18.72%; oleic, 27.68%; linoleic, 4.73% and linolenic, 1.77%. The results showed that bighorn sheep milk fat contained a smaller proportion of short-chain fatty acid than did the domestic sheep milk fat.

(4) Starch-gel electrophoretic analysis of the various sheep milk casein samples showed the presence of α_s- and β-casein. A pre-α_s zone was observed in two of the casein samples. When 2-mercaptoethanol was used in the buffer, the k-casein complex

was resolved into several diffuse zones. Domestic sheep milk appeared to contain no k-casein component.

(5) Polyacrylamide gel electrophoretic analysis of the casein and whey sample prepared from the various sheep milks indicated that α_s-casein, β-casein and β-lactoglobulin each contained two electrophoretically separable components.

(6) The average hexose, hexosamine and sialic acid contents of bighorn sheep milk casein was 0.40%, 0.16% and 0.15% respectively. The carbohydrate content of domestic sheep milk casein was somewhat lower than that of the bighorn sheep milk casein.

REFERENCES CITED

Alais, C. (1956)
Non-protein nitrogen substances (NPN) separated from the casein of cow milk by the action of rennet.
14th Intern. Dairy Congr., Rome 1956, Pt. 2. 823-836.
Cited from C. A. 51:15028 (1957)

Alais, C. (1963)
Release of non-protein nitrogen, phosphorus and sialic acid during the action of rennin on casein.
Ann. Biol. Anim. Biochim. Biophys. 3:391-404.

Alais, C. and Jolles, P. (1961)
Comparative study of the caseino-glycopeptides obtained after rennin digestion of the caseins of milk of cow, sheep and goat. II. Study of the non-peptide part.
Biochim. Biophys. Acta 51:315-322.

Anastassiadis, P. A. and Common, R. H. (1955)
The use of an ion-exchange resin for tissue hydrolysis in the determination of hexosamine.
J. Sc. Food Agric. 4:229-231.

Anastassiadis, P. A. and Common, R. H. (1958)
Liberation of hexosamine, hexuronic acid and hydrocyl proline from tissues by resin hydrolysis.
Can. J. Biochem. Physiol. 36:413-424.

Aschaffenburg, R. (1961)
Inherited casein variants in cow's milk.
Nature 192:431

Aschaffenburg, R. (1963)
Inherited casein variants in cow's milk. II.
Breed differences in the occurrence of beta-casein variants.
J. Dairy Res. 30:251-258.

Aschaffenburg, R. (1964)
Protein phenotyping by direct polyacrylamide-gel electrophoresis of whole milk.
Biochim. Biophys. Acta 82:188-191.

Aschaffenburg, R. (1965)
Symposium: Milk proteins. Variants of milk proteins and their pattern of inheritance.
J. Dairy Sci. 48:128-132.

Aschaffenburg, R. and Drewry, J. (1955)
Occurrence of different beta-lactoglobulin in cow's milk.
Nature 176:218-219.

Aschaffenburg, R. and Drewry, J. (1957a)
Genetics of beta-lactoglobulin of cow's milk.
Nature 180:376-378.

Aschaffenburg, R. and Drewry, J. (1957b)
Improved method for the preparation of crystalline beta-lactoglobulin and alpha-lactalbumin from cow's milk.
Biochem. J. 65:273-277.

Ashton, W.M., Owen, J.B. and Ingleton, J.W. (1964)
A study of the composition of clun forest ewe's milk.
J. Agric. Sci. 63:85-90.

Association of Official Agricultural Chemists (1960)
Official methods of analysis. 9th ed., Washington, D.C.

Bailey, G.L. (1952)
Studies on variations in the solids-not-fat content of milk. II. Variation due to stage in lactation.
J. Dairy Res. 19:102-108.

Baker, B.E., Huang, F. Y-Y. and Harington, C.R. (1963)
The carbohydrate content of polar bear milk casein.
Biochemical and Biophysical Research Communications 13:227-230.

Barnicoat, C.R., Murray, P.F., Roberts, E.M. and Wilson, G.S. (1956)
Milk secretion studies with New Zealand Romney ewes. Parts V-XI.
J. Agric. Sci. 48:9-35.

Bartley, E.E., Zaletel, J.H., Bird, E.W., Cannon, C.Y., Wise, G.H. and Kempthorne, O. (1951)
The relationship of stage of lactation, pasturage and environmental temperature to the degree of unsaturation of cow milk fat.
J. Dairy Sci. 34:536-547.

Beeby, R. and Nitschmann, H.S. (1963)
The action of rennin on casein. The disruption of k-casein complex.
J. Dairy Res. 30:7-16.

Bell, K. (1962)
One-dimensional starch-gel electrophoresis of bovine skimmilk.
Nature 195:705-706.

Bhattacharya, S.D., Raychoudhuay, A.K., Sinha, N.K. and Sen, A. (1963)
Inherited alpha-lactalbumin and beta-lactoglobulin polymorphism in Indian Zebu cattle. Comparison of Zebu and Buffalo alpha-lactalbumin.
Nature 197:797-799.

Bills, D.D., Khatri, L.L. and Day, E.A. (1963)
Method for the determination of the free fatty acids of milk fat.
J. Dairy Sci. 46:1342-1347.

Blood, D.A. 1965
(Personal communication)

Blumberg, B.S, and Tombs, M.P. (1958)
Possible polymorphism of bovine alpha-lactalbumin.
Nature 181:683-684.

Bonnier, G., Hansson, A. and Jarl, F. (1946)
Studies in the variation of calory content of milk.
Acta Agr. Snecana 2:159-169.

Brunner, J.R. and Thompson, M.P. (1959)
Some characteristics of glyco-macropeptide of casein -- a product of the primary rennin action.
J. Dairy Sci. 42:1881-1883.

Cayen, M.N., Henneberry, G.O. and Baker, B.E. (1962)
Studies on casein. IV. The sialic acid content of casein.
J. Dairy Sci. 45:706-709.

Cowan, I. McI. (1940)
Distribution and variation in the native sheep of North America.
Amer. Midl. Nat. 24:505-580.

Cowan, I. McI. (1944)
Report of wildlife studies in Jasper, Banff and Yoho National Parks.
Canadian National Parks Bureau Report.

Craig, B.M. and Murty, N.L. (1958)
The separation of saturated and unsaturated fatty acid esters by gas-liquid chromatography.
Can. J. Chem. 36:1297-1301.

De Koning, P.J., Jenness, R. and Wijnand, P. (1963)
Some aspects of the determination of N-acetylneuraminic acid, and its use for comparison of the action of heat and rennin on casein.
Neth. Milk and Dairy J. 17:352-363.

DeMan, J.M. (1961)
Gas chromatography of the short-chain fatty acids of milk fats.
Milchwissenschaft 16:245-247.

DeMan, J.M. (1964)
Determination of the fatty acid composition of milk fat by dual-column temperature-programmed gas-liquid chromatography.
J. Dairy Sci. 47:456-457.

Dimick, P.S. and Patton, S. (1965)
Structure and synthesis of milk fat VII. Distribution of fatty acids in milk fat triglycerides with special reference to butyrate.
J. Dairy Sci. 48:444-449.

Downing, S.C. (1947)
Mammals of the mountains.
Canadian Nat. 9:30-31.

Dreizen, P., Noble, R.W. and Waugh, D.F. (1962)
Light scattering studies of alpha-S1, 2-caseins.
J. Am. Chem. Soc. 84:4938-4943.

El-Sokkary, A.M., Sirry, I. and Hassan, H.A. (1949)
Composition and variation of the milk of Egyptian sheep.
J. Agr. Sci. 39:287-293.
Cited from C. A. 48:3585.

Fish, J.C. (1957)
Activity and specificity of rennin.
Nature 180:345.

Flook, D.R. (1964)
Range relationships of some ungulates native to Banff and Jasper National Parks, Alberta.
Grazing in Terrestrial and Marine Environments.
Fourth Symposium of British Ecological Society. pp. 119-128.

Fox, K.K. (1958)
Separation of a calcium-soluble fraction of casein from isoelectric casein.
J. Dairy Sci. 41:715.

Gander, G.W., Jensen, R.G. and Sampugna, J. (1962)
Analysis of milk fatty acid by gas-liquid chromatography.
J. Dairy Sci. 45:323-328.

Gehrke, C.W., Freeark, C.W., Oh, Y.H. and Chun, P.W. (1964)
Isolation of electrophoretically pure beta-caseins.
Anal. Biochem. 9:423-430.

Godden, W. and Puddy, C.A. (1935)
The yield and composition of the milk of the ewe.
J. Dairy Res. 6:307-312.

Gordon, W.G. and Basch, J.J. (1963)
Differences in the amino acid composition of genetic variants of bovine α_s-casein.
Federation Proc. 22:657.

Gordon, W.G., Basch, J.J. and Kalan, E.B. (1960)
Difference in amino acid composition of beta-lactoglobulins A and B.
Biochem. Biophys. Res. Comm. 3:672-674.

Gordon, W.G., Basch, J.J. and Kalan, E.B. (1961)
Amino composition of β-lactoglobulins A, B and AB.
J. Biol. Chem. 236:2908-2911.

Gordon, W.G. and Semmett, W.F. (1953)
Isolation of crystalline α-lactalbumin from milk.
J. Am. Chem. Soc. 75:328-330.

Green, H. U. (1949)
The bighorn sheep of Banff National Park.
Canada Dept. Resources and Development, National Park and Historic Sites Service.

Hall, E. R. and Kelson, K. R. (1959)
The Mammals of North America. Vol. II.
Ronald Press, New York. p. 1030-1032.

Hilditch, T. P. (1956)
The Chemical Constitution of Natural Fats.
Chapman and Hall, London. p. 138.

Hipp, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. (1950)
Separation of gamma-casein.
J. Am. Chem. Soc. 72:4928-4931.

Hipp, N. J., Groves, M. L., Custer, J. H. and McMeekin, T. L. (1952)
Separation of alpha-, beta- and gamma-casein.
J. Dairy Sci. 35:272-281.

Hipp, N. J., Groves, M. L. and McMeekin, T. L. (1959)
Separation of the components of alpha-casein.
The preparation of alpha-3-casein.
Abstr. No. 12, 136th meeting, Am. Chem. Soc.
Sept. 1959.

Huang, F. Y-Y. and Baker, B. E. (1964)
Casein. VI. Determination of sialic acid in casein.
J. Sci. Food Agric. 15:312-315.

Huang, F. Y-Y., Henneberry, G. O. and Baker, B. E. (1964)
Studies on casein. VII. The carbohydrate constitution of Glycopeptideic material isolated from enzymic hydrolysated of k-casein.
Biochim. Biophys. Acta 83:333-338.

Iotov, I. and Ikonomov, L. (1957)
Composition and properties of milk from Tsigai ewes at a Bulgarian experimental farm.
Dairy Sci. Abstr. 19:1035-1036.
Cited from C. A. 53:15398-(1959)

James, A. T. and Martin, A. J. P. (1956)
Gas-liquid chromatography: the separation and identification of the methyl esters of saturated and unsaturated acids from formic acid to n-octadecanoic acid.
Biochem. J. 63:144-152.

Jankowski, Z. (1953)
The chemical composition of sheep milk and whey.
Roczniki Panstwowego Zakladu Hig. 1953, 459-471.
Cited from C. A. 48:7808 (1954).

Jenness, R., Larson, B. L., McMeekin, T. L., Swanson, A. M., Whitnah, C. H. and Whitney, R. McL. (1956)
Nomenclature of the protein of bovine milk.
J. Dairy Sci. 39:536-541.

Jenness, R. and Patton, S. (1959)
Principles of Dairy Chemistry.
Chapman and Hall, London.
pp. 5-22.

Jensen, R. G., Sampugna, J. and Gander, T. W. (1962)
Fatty acid composition of the lipids from pooled raw milk.
J. Dairy Sci. 45:329-331.

Johansson, B. and Svennerholm, L. (1956)
The content of carbohydrate in caseins from different species.
Acta Physiologica Scandinavia 37:324-329.

Jolles, P., Alais, C. and Jolles, J. (1962)
Amino acid composition of k-casein and terminal amino acids of k- and para-k-casein.
Arch. Biochem. Biophys. 98:56-57.

Kalan, E. B., Greenberg, R., Thompson, M. P. and Pepper, L. (1963)
Some chemical characteristics of α_s-caseins.
Federation Proc. 22:657.

Kalan, E. B., Greenberg, R., Walter M. and Gordon, W. G. (1964)
Chemical properties of beta-lactoglobulins A, B and C.
Biochem. Biophys. Res. Comm. 16:199-203.

Kiddy, C. A., Johnston, J. O. and Thompson, M. P. (1964)
Genetic polymorphism in caseins of cow's milk.
I. Genetic control of alpha-S-casein variation.
J. Dairy Sci. 47:147-151.

Kon, S.K. and Cowie, A.T. (1961)
Milk: The mammary gland and its secretion.
Academic Press, New York and London.
Vol. II. pp. 230-244.

MacRae, H.F. and Baker, B.E. (1958)
Application of electrophoresis on paper to the estimation of alpha-, beta-, and gamma-casein.
J. Dairy Sci. 41:233-240.

Macy, I.G., Kelly, H.J. and Sloan, R.E. (1953)
The composition of milk.
Nat. Res. Council Pub. 254

Malpress, F.H. (1961)
Sialic acid release by rennin action on cow and human milk.
Biochem. J. 80:19P.

Manson, W. (1961)
The N-terminal amino acid residue of alpha-s-casein.
Arch. Biochem. Biophys. 95:336-342.

Marier, J.R., Tessier, H. and Rose, D. (1963)
Sialic acid as an index of the k-casein content of bovine skimmilk.
J. Dairy Sci. 46:373-379.

McMeekin, T.L., Groves, M.L. and Hipp, N.J. (1957)
The separation of a new component of casein.
Abstr. No. 143, 131st meeting, Am. Chem. Soc.
April 1957.

McMeekin, T.L. (1954)
Milk proteins.
Academic Press, New York.
The Proteins. Vol. II. Pt. A.

McKenzie, H.A. and Wake, R.G. (1961)
An improved method for the isolation of k-casein.
Biochim. Biophys. Acta 47:240-242.

McKinley, W.P. and Read, S.I. (1961)
Use of starch-gel electrophoresis to illustrate inhibition of rabbit liver esterases by organo-phosphorus pesticides.
J.A.O.A.C. 44:726-730.

Mellander, O. (1939)
Electrophoretic studies on casein.
Biochem. Z. 300:240-245 (1939)
Cited from C. A. 33:4278 (1939).

Montgomery, R. (1961)
Further studies of the phenol-sulfuric acid reagent for carbohydrate.
Biochim. Biophys. Acta 48:591-593.

Morr, C.V. and Kenkare, D.B. (1964)
On the heterogeneity of beta-lactoglobulin.
J. Dairy Sci. 47:294-295.

Murthy, G.K. and Whitney, R.M. (1958)
A comparison of some of the chemical and physical properties of gamma-casein and immune globulins of milk.
J. Dairy Sci. 41:1-12.

Nejim, H.T. (1963)
The composition of Iraqi sheep's milk.
J. Dairy Res. 30:81-85.

Nitschmann, H., Wissmann, H. and Henzi, R. (1957)
Uber ein glyto-makropeptid, ein spaltprodukt das caseins, enhralt en durch Einwirkung Von lab.
Chimia 11:76-77.

Ogston, A.G. and Tombs, M.P. (1957)
The heterogeneity of bovine beta-lactoglobulin.
Biochem. J. 66:399-403.

Overman, O.R., Garrett, O.F., Wright, K.E. and Sanmann, F.P. (1939)
Composition of milk of brown Swiss cows.
III. Agr. Exp. Sta. Bull. 457.

Parrish, D.B., Wise, G.H., Hughes, J.S. and Atkeson, F.W. (1948)
Properties of the colostrum of the dairy cow. II. Effect of prepartal rations upon the nitrogenous constituents.
J. Dairy Sci. 31:889-895.

Parrish, D.B., Wise, G.H., Hughes, J.S. and Atkeson, F.W. (1950)
Properties of the colostrum of the dairy cow. V. Yield, specific gravity and concentrations of total solids and its various components of colostrum and early milk.
J. Dairy Sci. 33:457-465.

Patton, S., McCarthy, R.D., Evans, L. and Lynn, T.R. (1960)
Structure and synthesis of milk fat. I. Gas chromatography analysis.
J. Dairy Sci. 43:1187-1195.

Pedersen, K.O. (1936)
Ultracentrifugal and electrophoretic studies on the milk proteins. I. Introduction and preliminary studies with fractions from skimmilk.
Biochem. J. 30:948-960.

Perrin, D.R. (1958)
The chemical composition of the colostrum and milk of ewe.
J. Dairy Res. 25:70-74.

Perry, N.A. and Doan, F.J. (1950)
A picric acid method for the simultaneous determination of lactose and sucrose in dairy products.
J. Dairy Sci. 33:176-185.

Piez, K.A., Davie, E.W., Folk, J.E. and Gladner, J.A. (1961)
Beta-lactoglobulin A and B. I. Chromatographic separation and amino acid composition.
J. Biol. Chem. 236:2912-2916.

Pilson, M.E.Q., Henneberry, G.O. and Baker, B.E. (1960)
Studies on casein. III. Preparation of carbohydrate-rich fraction and a calcium-sensitive fraction from alpha-casein.
J. Sci. Food Agric. 11:640-644.

Plowman, R.D., Townend, E.R., Kiddy, C.A. and Timasheff, S.N. (1959)
Inheritance of the beta-lactoglobulins in cow's milk.
J. Dairy Sci. 42:922

Prekopp, I. (1957)
Sheep's milk and its importance in the dairy industry.
Vyziva Lidu 12:46 (1957)
Cited from C. A. 54:25355 (1960).

Robbins, R.M. and Kronman, M.J. (1964)
A simplified method for preparing alpha-lactalbumin and beta-lactoglobulin from cow's milk.
Biochim. Biophys. Acta 82:186-188.

Rose, D. and Mariérr, J.P. (1963)
Fractionation of k-casein on a diethylaminoethyl cellulose column.
J. Dairy Sci. 46:1323-1328.

Samuels, E.R., Coffin, A., Julien, J.P. and Baker, B.E. (1960)
Studies on milk powders. IV. The foam and sediment fractions of reconstituted whole milk.
J. Dairy Sci. 43:624-629.

Sartore, G. and Lai, P. (1957)
Some physico-chemical properties of Sardinian sheep milk and their variations.
Studi Sassaresi, Seizione III. 5. 142-151.
Cited from C. A. 53:19393 (1959).

Schmidt, D.G. and Payens, T.A.J. (1963)
The purification and some properties of a calcium-sensitive alpha-casein.
Biochim. Biophys. Acta 78:492-499.

Sloan, R.E., Jenness, R., Kenyon, A.L. and Regehr, E.A. (1961)
Comparative biochemical studies of milks. 1. Electrophoretic analysis of milk proteins.
Comp. Biochem. Physiol. 4:47-62.

Smith, L.M. (1961)
Quantitative fatty acid analysis of milk fat by gas-liquid chromatography.
J. Dairy Sci. 44:607-622.

Smithies, O. (1955)
Zone electrophoresis in starch-gels: Group variations in the serum proteins of normal human adults.
Biochem. J. 61:629-641.

Smithies, O. (1959)
An improved procedure for starch-gel electrophoresis: Further variation in the serum proteins of normal individuals.
Biochem. J. 71:585-587.

Stoffel, W., Chu, F. and Ahrens, E.H. (1959)
Analysis of long-chain fatty acids by gas-liquid chromatography.
Anal. Chem. 31:307-308.

Sullivan, R.A., Fritzpatrick, M.M. and Stanton, E.K. (1959)
Distribution of kappa-casein in skimmilk.
Nature 183:616.

Svennerholm, L. (1957)
Quantitative estimation of sialic acids. II. A colorimetric resorcinol-hydrochloric acid method.
Biochem. Biophys. Acta 24:604-611.

Swaisgood, H.E. and Brunner, J.R. (1962)
Characterization of k-casein obtained by fractionation with trichloroacetic acid in concentrated urea solution.
J. Dairy Sci. 45:1-11.

Swaisgood, H.E. and Brunner, J.R. (1963)
Characteristics of kappa-casein in the presence of various dissociating agents.
Biochem. Biophys. Res. Comm. 12:148-151.

Swaisgood, H.E., Brunner, J.R. and Lillevik, H.A. (1964)
Physical parameters of k-casein from cow's milk.
Biochemistry 3:1616-1623.

Thompson, M.P., Kiddy, C.A., Pepper, L. and Zittle, C.A. (1962)
Variations in the alpha-s-casein fraction of individual cow's milk.
Nature 195:1001-1002.

Thompson, M.P. and Kiddy, C.A. (1964)
Genetic polymorphism in caseins of cow's milk. III. Isolation and properties of alpha-s1-casein A, B and C.
J. Dairy Sci. 47:626-632.

Thompson, M.P., Kiddy, C.A., Johnston, J.O. and Weinberg, R.M. (1964)
Genetic Polymorphism in caseins of cow's milk. II. Confirmation of the genetic control of beta-casein variation.
J. Dairy Sci. 47:378-381.

Thompson, M.P. and Pepper, L. (1964)
Some characteristics of alpha-s- and beta-casein variants.
J. Dairy Sci. 47:293-294.

Vogel, A. (1956)
Practical organic chemistry.
Third edition. P. 169-179.
Longmans, Green, New York.

Von Hippel, P.H. and Waugh, D.F. (1955)
Casein: Monomers and polymers.
J. Am. Chem. Soc. 77:4311-4319.

Wake, R.G. and Baldwin, R.L. (1961)
Analysis of casein fractions by zone electrophoresis in concentrated urea.
Biochim. Biophys. Acta 47:225-239.

Warner, R.C. (1944)
Separation of alpha- and beta-casein.
J. Am. Chem. Soc. 66:1725-1731.

Warren, L. (1959)
The thiobarbituric acid assay of sialic acids.
J. Biol. Chem. 234:1971-1975.

Waugh, D.F. and Von Hippel, P.H. (1956)
Kappa-casein and the stabilization of casein micelles.
J. Am. Chem. Soc. 78:4576-4582.

Waugh, D.F., Ludwig, M.L., Gillespie, J.M., Melton, B., Foley, M. and Kleiner, E.S. (1962)
The alpha-s-caseins of bovine milk.
J. Am. Chem. Soc. 84:4929-4938.

Zittle, C.A. (1962)
Procedure for isolation of k-casein by use of sulfuric acid.
J. Dairy Sci. 45:650

Zittle, C.A. and Custer, J.H. (1963)
Purification and some of the properties of alpha-s-casein and k-casein.
J. Dairy Sci. 46:1183-1188.