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Destructive Distillation of Casein in a Partial Vacuum.

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According to recent views, proteins are made up of heterocyclic complexes known to yield cyclic anhydrides when partially hydrolyzed, and amino acids when completely hydrolyzed. Since the character of the decomposition products seems to depend upon the nature of the degradation process, the desirability of investigating the products formed by methods other than hydrolytic is apparent.

The intention in the present work was to adapt the methods of Johnson and Daschavsky[1, 2] to casein and, as far as possible, to obtain quantitative information in regard to the products of decomposition. Casein was selected as the basic material because it may readily be obtained in quantity. It was also desired to make a comparison between this relatively complete protein and silk fibroin.

The distillation was conducted in an iron pipe 24 inches long and 3½ inches in diameter. The pipe was fitted at each end with an iron screw cap. An iron delivery tube, 20 inches long and ¾ inch in diameter, was threaded into the center of one of the caps. The screw caps were rendered gas tight by coating the threads with white lead. Graphite proved to be unsatisfactory for this purpose.

Five hundred gram portions of white, finely powdered, commercial casein were heated in the iron pipe on an ordinary combustion furnace for each of the six runs. By slightly tilting the furnace, the molten material was prevented from clogging the delivery pipe. For collecting the liquid and gaseous products, 10 round bottom, side neck, Pyrex flasks were connected in series to the delivery tube. A manometer and a good water pump were attached to the terminal flask. An oil pump was used in some of the experiments but it was found to be unsatisfactory due to its failure to maintain a constant vacuum. Flasks 3, 4, and 5, containing a total of 450 cc. of approximately 3 N sodium hydroxide, caught the acidic gaseous products. Flask 5 was a safety flask. Flasks 7, 8, and 9, containing a total of 500 cc. of approximately 3 N sulfuric acid, caught the volatile basic products. Flask 10 was a safety trap.

In each of the six runs, the pipe and flasks were evacuated to about 25 mm. This pressure was maintained for about 9 hours. The pipe was first heated gently for 1½ hours, then more vigorously, and finally as strongly as possible. The visible products of

the distillation collected in the following order: water vapor, white fumes, a white precipitate in the alkali, a yellow liquid, a black liquid, and a red liquid in the acid. Gases were still issuing from the pipe when the heating was discontinued.

As may be seen from the data given in Table I the weights of products formed are comparable to those obtained by Johnson and Daschavsky. It should be noted, however, that the weights of volatile products reported by these authors were not obtained directly but by difference.

TABLE I.

Products	Weights of products recovered								Products obtained by Johnson and Daschavsky
	Ia	II	III	IV	V	VIb	Total		
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	%	%
Heavy liquids	137.4	132.5	143.4	148.0	144.8	121.8	827.9	27.6	
Light liquids	48.7	47.5	26.3	42.7	27.2	22.3	214.7	7.2	43.0
Gases soluble in alkali	95.2	85.8	67.5	73.4	59.6	49.7	431.2	14.4	15.9c
Gases soluble in acids	22.2	14.8	12.6	37.4	29.0	10.4	126.4	4.2	
Residual coke	183.7	161.6	159.3	150.0	157.3	285.0	1096.9	36.5	41.0
Total	487.2	442.2	409.1	451.5	417.9	489.2	2697.1	89.9	99.9

a—Pressure rose gradually to 95 mm. Relatively low temperature was maintained throughout.

b—Relatively low temperature and about 25 mm. pressure were maintained throughout.

c—Obtained by subtracting the sum of weights of liquids and coke from the original weight of protein taken.

Qualitative tests of the sulfuric acid solution indicated the presence of ammonia and primary aliphatic amines. Ammonia was liberated when a portion of the acid solution was neutralized with sodium hydroxide and distilled. The Rimini and carbylamine reactions were used to test for amines.

CO_2, present in the alkaline solution chiefly as sodium bi-carbonate, was determined quantitatively. Barium carbonate, precipitated by the addition of an excess of barium hydroxide to 10 cc. of the alkaline solution, was washed and transferred to a two neck flask. The CO_2, liberated by the addition of 60 cc. of 6 N sulfuric acid, was collected in 50 cc. of 0.1559 N barium hydroxide by aspirating for 1 hour at 75° C., with a stream of air freed from CO_2. A correction was made for the CO_2 present in all reagents.

The 10 cc. of alkaline solution were found to contain 0.0374 g. of CO_2, the equivalent of 16.82 g. in the total volume. This weight of CO_2 is 3.1 per cent by weight of the alkali soluble gases and 0.56 per cent of the original casein.

A similar analysis was made using an aliquot portion of the original alkaline solution instead of the barium carbonate precipitate. By this analysis 19.09 g. of volatile acids, calculated as CO_2, were found. Assuming that acetic acid is the volatile acid present in addition to CO_2, 3.09 g. of acetic acid would be present in the total volume of alkaline solution. This weight is equivalent to 0.10 per cent of the original casein.

Quantitative determinations of other products will not be possible until a later time.

This is a preliminary report.

[1] Johnson, T. B., and Daschavsky, P. G., *J. Am. Chem. Soc.*, 1919, xli, 1147.
[2] Johnson, T. B., and Daschavsky, P. G., *J. Biol. Chem.*, 1924, lxii, 197.

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Microbic Dissociation III. B. C. G. (Bacillus Calmette-Guérin).

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We shall describe the dissociation of a tubercle bacillus of low virulence, originally of bovine origin, which was isolated by Calmette in 1908, and passed through 230 subcultures on a special bile medium. It is claimed that at the time of isolation it produced lesions in rabbits, but after long cultivation it has lost its pathogenicity for small laboratory animals. The organism is designated as "B.C.G." (Bacillus Calmette-Guérin) and for the last five years has been extensively used for vaccinating children, with the object of attaining prophylactic immunization.

The cultures in our possession were obtained from three different sources. The first was received from Dr. Watson of Ottawa, Canada, and the second was brought from Paris by Dr. Lawrason Brown in the spring of 1926. After seeding this second culture on Sauton's fluid medium, growth and cultural differences were noticed in some of the flasks, and it was dissociated into two distinct types of colonies.

However, the study reported here on dissociation was carried out with the third culture, which came direct from the Pasteur Institute, sent to us by Professor Calmette. As soon as it was received it was suspended in salt solution and filtered as described previously,[1] and inoculated on the surface of gentian-violet medium plates. After a