The Transformation of Proteins into Fats during the Ripening of Cheese. (Preliminary Communication.)

By M. Nierenstein.

(Communicated by Dr. Leonard Hill, F.R.S. Received November 8, 1910,—Read January 19, 1911.)

(From the Chemical Department, University of Bristol.)

It is generally accepted that the so-called ripening of cheese is accompanied by an increase in the amount of fat, and this statement is repeatedly brought forward as one of the evidences that proteins serve as a source for the formation of fat in the animal body. The production of fat during the ripening of cheese is traced to bacteria and other fungi, which break down the proteins present and set free fat, together with other degradation products.*

During some previous investigation, jointly with Dr. Breinl,† we had reason to doubt the accuracy of the view that proteins were the precursors of fat in the organism, and it appeared to me that, as the argument was partly based on a corresponding change during the ripening of cheese, this change was worth re-investigation.

The older investigators dealt either with increase of fat and decrease of casein during the ripening process, or else with the increase of fat only. Blondeau,‡ in 1869, examined Roquefort cheese, and his striking results are as follows :—

	Fresh.	1 month.	2 months.	1 year.
	Per cent.	Per cent.	Per cent.	Per cent.
Casein	85·43	61·33	43·28	40·23
Fat	1·85	16·12	18·30	18·33

But these results have so far never been confirmed.

Stohmann,§ who carefully repeated this work, and who is an authority on the chemistry of milk, concludes that "das Krasseste aber, was in diesen

* See Leonard Hill, 'Recent Advances in Physiology and Bio-chemistry' (1908), p. 290; F. B. Leathes, 'The Fats' (1910), p. 106; and also E. Abderhalden, 'Physiologische Chemie' (1909), p. 436.

† A. Breinl and M. Nierenstein, 'Zeitschr. f. Immunitätsforschung,' 1909, vol. 2 p. 169.

‡ Blondeau, 'Ann. Chim. Physique' (4), vol. 1, p. 208.

§ Stohmann, 'Milch u. Molkereiprodukte' (1898), p. 842.

Analysen geleistet wird, besteht in der Angabe, nach welcher der aus Schafmilch bereitete Käse im frischen Zustande nur 1·85 pro cent. Fett enthalten soll. Diese als Ausgangspunkt der Vergleichung geltende Zahl ist so ungeheuerlich, dass kein weiteres Wort über dieselbe zu verlieren ist."

Nadina Sieber,* who was also unable to confirm Blondeau's experiment, concludes, "dass Blondeau Dichtung für Wahrheit gern gelten lässt," and that "die Analysen gar nicht gemacht, sondern erfunden worden sind."

Brassier,† in 1869, made a further study of this question, and showed that the ripening of cheese actually results in a decrease of both the fat and proteins. But these results were not confirmed by either Müller‡ or Kellner.§

More recent, and also more conclusive, is the work of Jacobstahl|| and of Windisch,¶ who showed that during the ripening process the percentage of the ether extract increased, but both failed to show a decrease in the proteins.

In all this work one fact stands out prominently, viz., that all the investigators evaporated down the ethereal extract from cheese and *without further investigation* weighed the residue as "fat."

Now it seemed to me more than likely that the ethereal solution might contain some other substance or substances formed during the ripening process, and that the apparent increase of fat was actually due to such products. For this reason I examined a large amount of fat extracted by ether from an old Cheddar cheese. The results I obtained showed that, besides fat, the following substances were present:—

1. Free cholesterol,
2. Cadaverine,
3. Putrescine,
4. Aminovaleric acid.

I conclude from this observation that it is not legitimate to consider that the increase in weight of the ethereal extract obtained from cheese during the ripening process affords any evidence that proteins serve as a source for fat formation in cheese, and preliminary quantitative experiments clearly showed that the actual amount of these products present with the fat was sufficient to account for the increase in the weight of the ether extract observed by so many investigators.

Any argument, then, based upon this, that proteins serve as a source for fat-formation in the animal body, falls to the ground.

* Nadina Sieber, 'Journ. f. prakt. Chemie' (2), vol. 21, p. 203 (1880).

† Brassier, 'Ann. Chim. Physique' (4), vol. 5, p. 270.

‡ Müller, 'Landwirtsch. Jahrbücher,' vol. 1, p. 68.

§ Kellner, 'Landwirtsch. Versuchsstationen,' vol. 25, p. 39.

|| Jacobstahl, 'Pflüger's Archiv,' vol. 54, p. 484.

¶ Windisch, 'Arbeiten aus dem Kaiserlichen Gesundheitsamt,' vol. 17, p. 283.

Experimental Part.

6874 grm. of a Cheddar cheese, about four years old, were extracted with ether in a Soxhlet apparatus. The ethereal solution was evaporated to dryness, the residue dissolved in ether and precipitated with acetone, the precipitate collected, and the process repeated four times. The different ether and acetone solutions were collected and examined separately.

The precipitate (17·5 grm.), which contained traces of nitrogen, was redissolved in ether and once more precipitated with acetone, but only the precipitate formed *at once* was collected. The product thus obtained was free from nitrogen. When dissolved in warm chloroform it separated on cooling in feather-like crystalline needles, melting at 139° to 140° (cholesterol melts at 147°), and gave all reactions for cholesterol. But in order to further confirm the presence of this substance, the benzoyl derivative was prepared; this crystallised from alcohol in octahedra, which melted at 176° to 177°, and gave on analysis the following data:—

0·1527 grm. gave 0·4654 grm. CO_2 and 0·1399 grm. H_2O.
$C_{27}H_{45}O{\cdot}C_7H_5O$ requires C = 83·20, H = 10·20.
Found C = 83·12, H = 10·18.

All the acetone-ether solutions were collected and evaporated to dryness, and the residue treated in the cold with sodium carbonate and saturated with carbon dioxide. When carefully acidified with dilute sulphuric acid the filtrate gave a white precipitate which contained nitrogen. The melting point of this substance, crystallised from either benzene or alcohol and methylated spirit, was between 162° and 229°, showing that it was not pure. It was purified by dissolving 1 grm. of the material in 40 c.c. of absolute alcohol and adding 5 c.c. of boiling benzene to the solution. On cooling, well-defined needles were formed, which melted at 246° to 251°, and gave the following data on analysis:—

0·2731 grm. gave 0·5116 grm. CO_2 and 0·2334 grm. H_2O.
0·1881 „ 19·5 c.c. N_2 (19°, 758 mm.).
$C_5H_{11}O_2N$ (aminovaleric acid) requires C = 51·28, H = 9·40, N = 11·95.
Found C = 51·09, H = 9·36, N = 12·07.

The benzoyl derivative prepared by Fischer's method formed small prismatic needles (m. p. 173°—179°), which gave the following data on analysis:—

0·1872 grm. gave 0·4556 grm. CO_2 and 0·1139 grm. H_2O.
0·1531 „ 8·5 c.c. N_2 (16°, 756 mm.).
$C_{12}H_{15}O_3N$ requires C = 65·16, H = 6·78, N = 6·33.
Found C = 64·93, H = 6·81, N = 6·55.

These data point to the fact that the ethereal extract contained an acid of the aminovaleric acid type, but neither the melting point of the free acid nor that of the benzoyl derivative corresponds with any of the known acids.

As the different aminovaleric acids have lately been carefully studied by Slimmer,* in Emil Fischer's laboratory, I presume that the product I obtained was probably a mixture of two or three aminovaleric acids, especially if one considers that the melting points of both the free acid and the benzoyl derivative were not sharp.

After repeatedly washing the previously mentioned extract with sodium carbonate, the residue was freed from this salt by washing with water and then treated in the cold with a known amount of N/10 hydrochloric acid The solution obtained in this manner was then carefully neutralised with N/10 potassium hydroxide and evaporated nearly to dryness. The product was then treated with boiling alcohol and purified by means of the mercury-compound. The free bases were almost entirely soluble in alcohol, and on treating the solution with concentrated hydrochloric acid the hydrochloride of putrescine (tetramethylenediamine) crystallised out.

The chloroplatinate, picrate, and dibenzoyl derivative all agreed in their properties with the corresponding derivatives of that base. Since putrescine has been found in Emmenthal cheese,† and also, as will be shown in a further communication, in Cheddar cheese, the presence of putrescine in the cheese-fat ethereal extract may be regarded as certain.

After the putrescine hydrochloride had separated out from the alcoholic solution—a process which, according to Ackermann,‡ is quantitative—the filtrate was concentrated to about one-third of its volume, when the hydrochloride of cadaverine (pentamethylenediamine) crystallised out. The free base, the chloroplatinate, picrate, and dibenzoyl derivative were prepared, and found to correspond in all their properties with the base and its derivatives.

The presence of cadaverine in cheese has not yet been reported.

* Slimmer, 'Ber. Deutsch. Chem. Ges.,' vol. 35, p. 400.

† Winterstein u. Thoeni, 'Zeitsch. f. physiol. Chemie,' vol. 36, p. 28; Winterstein, *ibid.*, vol. 41, p. 485. Putrescine has also been found in American Cheddar cheese (Van Slyke and E. B. Hart, 'Amer. Chem. Journ.,' vol. 29, p. 382).

‡ Ackermann, "Die Isolierung von Fäulnissbasen," 'Handbuch der Biochemischen Arbeitsmethoden (Abderhalden),' vol. 2, p. 1002.