

**NOTE ON A RECENT PAPER BY R. STEINEGGER ON THE "ALDEHYDE
FIGURE" OF MILK.**

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WE have been induced to refer to this paper by the fact that the method (ANALYST, xxxi., 46) proposed is likely to be of considerable use.

The author first shows that formalin added to milk hinders and finally prevents curdling by rennet. Next he discusses the increase of acidity on the addition of formalin pointed out by Hanne and Hesse, and shows that the acidity increases with the amount of formalin used and attains a maximum when about 5 per cent. (at least 1·8 per cent. of formaldehyde) is added, whether the addition is made all at once or in successive amounts.

He then gives the variations in the milk of *single cows*, and it is not pointed out in the abstract that the maxima and minima given are in the milk of single cows. Next he shows that the increase of acidity is not due to the action of a ferment causing oxidation to formic acid, as had been suggested by Hesse, and that it remains constant whether rennet is added to the milk or not. In addition to giving figures that show that the aldehyde figure is proportional to the total nitrogen in the milk, and that the total nitrogen (and therefore the percentage of proteids) can be calculated from the aldehyde figure, he gives some experiments with tyrosine and leucine, which show that the acidity is produced by the conversion of the amino groups into methylene-amino groups by condensation, with the consequent conversion of the amphoteric nature of the amino-acid into an acid one.

We think this paper important because—

1. The method appears to give an approximate estimation of the amino-nitrogen of the proteids.

2. It gives an indirect but very easy method for proteid estimation.

3. It adds another figure to those which can be easily determined in milk analysis, and thus will yield corroborative evidence.

Our first experiments were made with a view to see if we could obtain definite evidence of the combination with the amino-groups, and experimented with whey produced by the action of rennet and acid respectively.

Our results were :

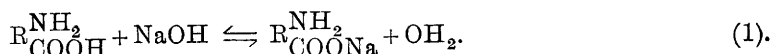
	Treated with Benzene-diazonium Chloride.	Diazotized and combined with β -naphthol.
Acid whey	Dark red colour	Bright yellow precipitate
Acid whey + CH_2O ...	Dark yellow colour	No reaction
Rennet whey	Bright red colour	Light yellow colour
Rennet whey + CH_2O ...	Light yellow colour	No reaction

In each case the wheys effervesced in the cold on treatment with nitrous acid, whilst after treatment with formaldehyde there was no gas given off in the cold. The amount of gas given off from the rennet whey corresponded to 0.013 per cent. of amino-nitrogen, while the increase of acidity corresponded to about 0.01 per cent. of amino-nitrogen on the assumption that each molecule of NH_2 corresponded to one equivalent of acidity. These results show the presence of both aromatic and fatty amino groups in milk which condense with formaldehyde.

We next experimented on a casein solution containing 0.2596 gram nitrogen by Kjeldahl per 100 c.c.; this gave an aldehyde figure of 9.8 c.c. $\frac{\text{N}}{10}$ soda per 100 c.c., and combined with 0.0394 gram CH_2O per 100 c.c., which is equivalent to 13.2 c.c. $\frac{\text{N}}{10}$ alkali. Ten c.c. of this solution gave 0.1601 gram casein by precipitation with acetic acid, and 0.1608 gram after the addition of formaldehyde.

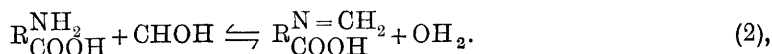
These results show that the "aldehyde figure" is not a complete measure of the amino-groups nor of the formaldehyde condensed.

Proteids certainly contain both amino and carboxyl groups, and when alkali is added (*e.g.*, soda) the following reaction takes place :



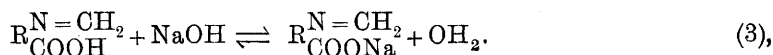
The point of neutrality to phenolphthalein is, on account of the presence of the basic NH_2 group, much nearer to the acid than to the sodium salt.

The action of formaldehyde is probably—



and the reaction in the presence of a large excess of formaldehyde is probably complete and very rapid.

The methylene-amino acid reacts with alkali thus :



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and the point of neutrality is probably very close to the salt on account of the feeble basicity of the methylene-amino group. The "aldehyde figure" measures the difference between the points of neutrality of the two reversible reactions (1) and (3).

It is therefore by no means certain that all alkalies will give the same "aldehyde figure." We have measured in four samples of milk the "aldehyde figure" with soda and strontia respectively. The results are expressed as c.c. N alkali per litre.

ALDEHYDE FIGURES WITH				
	Soda.			Strontia.
1.	17.4	20.2
2.	20.1	21.6
3.	18.8	20.2
4.	21.8	22.6

The strontia "aldehyde figure" is always higher than the soda figure, and the mean ratio is 1.1.

We also find in mixed milk that soda gives a mean "aldehyde figure" of 18.4, while strontia yields 20.2, also in the ratio of 1.1.

Steinegger, who used the Soxhlet-Henkel method of determining acidity, probably used soda as his alkali. We find that with soda 1 c.c. $\frac{N}{10}$ alkali corresponds to 0.0265 gram casein nitrogen, and 0.053 gram albumin nitrogen. Steinegger finds that on the average 1 c.c. $\frac{N}{10}$ alkali corresponds to 0.0303 gram of the nitrogen of milk (1 degree Soxhlet-Henkel = 2.5 c.c. $\frac{N}{10}$ alkali per 100 c.c. = 0.0758 gram N per 100 c.c.), whilst, taking the mean proportion of casein to albumin to be 7 : 1, we find 0.0298 gram using soda and 0.0271 gram using strontia.

As the titration is rather sharper with strontia than with soda, we prefer the use of the former solution.

The mean of 113 determinations gives 19.9 c.c. N strontia per litre of milk as the mean "aldehyde figure," with 22.6 c.c. as a maximum and 18.1 c.c. as a minimum. As showing the relative constancy in milk from a herd, we give the highest figures, the lowest figures, and a mean figure. All figures were on different dates:

	Aldehyde Figures.			Mean Solids-not-Fat.
Highest herd	21.0, 22.0, 22.0, 22.6	9.16
Mean herd	19.6, 20.2, 19.6, 19.6, 19.6	8.97
Lowest herd	18.1, 18.6, 18.1	8.61

We have also made a number of experiments on the change in the aldehyde figure when milk is acted on by micro-organisms, and on the amino groups in milk proteids. These will be discussed later.

