reception of the samples, small bulbs, with capillary tubes about 8 cm. long, similar to those used in the combustion of volatile liquids, were prepared. These bulbs were weighed empty, and were then filled with anhydrous hydronitric acid by inverting them in the acid, cooling the bulb with a sponge wet with liquid air, turning them into the upright position, freezing the acid with liquid air, sealing the capillary, and weighing again. Several attempts were made to ascertain the vapor density of the acid by placing one of these bulbs of the acid in the Victor Meyer tube, bringing the apparatus to a constant temperature, and then breaking the capillary of the bulb with two glass rods projecting down in the tube and so bent as to act like a pair of shears. In every case the acid exploded when the stem of the bulb was broken, the decomposition of 11 milligrams of the substance being sufficiently violent to pulverize the glass of both the inner and outer tubes of the apparatus. A successful determination was finally made by immersing the weighed and sealed bulb in liquid air until the acid was again frozen, then breaking off the tube and instantly dropping the bulb into the Victor Meyer tube that had already been brought to temperature equilibrium. A bath of boiling chloroform was employed.

0.0223 gram hydronitric acid gave 13.8 cc. gas at 747.6 mm. pressure and 19°, equivalent to 12.4 cc. at 760 mm. pressure and 0°.

	Calculated for HN_3	Found
Molecular weight	42.79 (H=2)	40.00
Weight per liter	1.923	1.798

This result shows that at a temperature only 25° above the boiling point of the acid, the compound has the molecular formula HN_3.

CORNELL UNIVERSITY,
November, 1906.

ON SOME PHENOMENA OBSERVED IN THE PEPTIC DIGESTION OF CASEINS.

BY J. H. LONG.

In following up the question of the relation of the casein from cow's milk to that from the goat,[1] I have determined some points in the behavior of the two on treatment with pepsin and very dilute hydrochloric acid. In each experiment I used 10 grams of pure air dry casein made by the Hammarsten method, and mixed with 500 milligrams of a very active specially prepared pepsin, furnished me by Armour & Co., and 1000 cc. of acid containing 2.33 grams of HCl. The mixtures were allowed to undergo digestion at a temperature of 38° in carefully cleaned Bohemian

[1] This Journal, **28**, 372.

glass flasks closed with stoppers with capillary openings. The flasks were cleaned by steaming and washing with the same dilute acid used in the experiments. A control flask was prepared in the same manner and filled with the dilute acid alone to be used for a purpose which will appear below. The mixtures were digested through a period of nearly two months, and were shaken up thoroughly several times each day. The object of this prolonged digestion was to give time for the formation of various cleavage products which might combine with the acid used in the reaction to form salt-like bodies, having definite effects on the titration values and the electrical conductivity of the mixtures. Accordingly from time to time portions were withdrawn from the flasks and titrated by the aid of phenolphthalein and dimethylaminoazobenzene. Tests of the conductivity were made on similar portions at each time of titration. As these tests were carried out side by side on each kind of casein, it was supposed that some points of difference might be disclosed.

Two samples of casein from cow's milk, made at different times, were used in the experiments, and these and the goat's milk casein will be designated as follows :—

A. First sample of cow casein.

B. Second sample of cow casein.

C. Goat milk casein.

In the first stages of the digestion, the casein mixed up readily with the acid liquid, but did not all dissolve. The residues which settled grew gradually less, however, and became lighter and more flocculent. This represents the so-called "pseudo nuclein," which in appearance and amount was characteristic for each casein. At the end of the digestion period this residue was filtered off, dried and weighed in each case, the volume of digesting liquid remaining being at the same time accurately measured. As the flasks were always thoroughly shaken before removing portions for the tests, these residue weights could be calculated to the basis of the whole original volume. The figures will be given below, but first the results obtained from time to time in the titration and conductivity tests of the digesting liquids must be shown.

The titration of the liquid was made always on 25 cc, using first the dimethylaminoazobenzene and $\frac{N}{10}$ sodium hydroxide. This measures the so-called "free" hydrochloric acid. Following this, phenolphthalein is added and then a further amount of alkali to distinct coloration. By this procedure, the "total" acid is measured and the end reading is always sharper than that secured in the presence of the dimethylaminoazobenzene alone. This total acid includes the actually uncombined hydrochloric acid as well as that loosely held by the cleavage products of the casein. This acidity must include finally that of certain organic acids

formed in the course of digestion by the gradual hydrolysis of the casein. Such acids are very weak in their behavior, but stronger than the phenolphthalein itself, considered as an acid. It is possible that only a relatively small portion of the acid bodies produced may be indicated in this manner, however.

The original hydrochloric acid, containing 2.33 grams per liter, was of such a strength that 25 cc. required for neutralization 16 cc. of $\frac{N}{10}$ sodium hydroxide, with phenolphthalein or the dimethylaminoazobenzene as indicator. In acid solutions of this strength titrations with the two indicators yield practically identical results. Therefore, since on mixing 1000 cc. of acid with 10 grams of casein there is in effect an increase in volume and a dilution we should expect to find on titration of 25 cc. of the acid mixtures something less than 16 cc. of the alkali required, assuming that on mixing the acid and casein, no immediate chemical change takes place. The figures in the table below show that this last assumption is not quite correct.

As soon as possible after mixing, portions were withdrawn from each flask for titration and conductivity tests. For the former the whole fluid was used and for the latter the supernatant liquid from 25 cc, obtained after giving the protein precipitate time to settle. It will be noticed that these first titrations, while approximately close to the 16 cc. for phenolphthalein, are already quite different for the other indicator. The conductivity tests show also a wide divergence from that of the acid alone, which was $\kappa_{20}=0.02347$. No dilution need be calculated here for this first conductivity test, since the casein had been allowed to settle before making it.

The tables given below show the extent of the immediate change in the condition of the acid and the factors which influence the conductivity. The values under the head of calculated conductivity are based on the amount of "free" hydrochloric acid as indicated by the titration with dimethylaminoazobenzene.

CASEIN A

Date	cc. of alkali for total acid.	cc. of alkali for "free" acid.	Conductivity found, κ_{20}.	Conductivity calculated, κ_{20}.
February 7	16.2	14.6	0.02060	0.02167
" 8	19.4	13.4	0.01850	0.01988
" 9	19.8	13.2	0.01780	0.01958
" 20	20.0	12.5	0.01630	0.01860
March 8	20.1	11.8	0.01558	0.01756
" 17	21.2	11.5	0.01408	0.01716
April 2	21.5	11.5	0.01364	0.01716

CASEIN B

Date	cc. of alkali for total acids.	cc. of alkali for "free" acid.	Conductivity found, κ_{30}	Conductivity calculated, κ_{20}
February 7	15.8	14.5	0.02070	0.02152
" 8	19.4	14.0	0.01865	0.01977
" 9	19.8	13.2	0.01800	0.01958
" 20	20.0	12.5	0.01659	0.01860
March 8	20.0	12.0	0.01523	0.01785
" 17	21.3	11.5	0.01409	0.01716
April 2	21.6	11.5	0.01372	0.01716

CASEIN C

Date	cc. of alkali for total acids.	cc. of alkali for "free" acid.	Conductivity found, κ_{20}	Conductivity calculated, κ_{20}
February 7	15.8	14.5	0.02079	0.02152
" 8	19.2	13.7	0.01900	0.02033
" 9	19.4	13.0	0.01806	0.01929
" 20	20.0	12.2	0.01676	0.01816
March 8	20.0	11.2	0.01555	0.01666
" 17	21.0	11.0	0.01454	0.01641
April 2	21.0	11.0	0.01401	0.01641

In the case of the mixture A, it is evident that a more marked modification had taken place in the casein before the test could be made than in the other cases. The phenolphthalein titration is already a little higher, while the dimethylaminoazobenzene titration indicates a decided loss of free acid by combination. This early discrepancy between the titrations with the two indicators is easily explained. There is evidently at once a superficial combination which does not prevent the acid from showing as "free" when phenolphthalein is employed, but which is still stable enough to make part of the acid appear as combined when the other indicator is used. The three caseins resemble each other closely at the outset in their behavior with dimethylaminoazobenzene but before the end of the series there is some divergence between the values of A and B on the one hand and C on the other. In all cases there is a rapid increase in the total acidity as measured by the one indicator and a correspondingly rapid decrease in the "free" acid as measured by the other. These numbers have some significance, as it will be noticed that the values for A and B are essentially the same, while for C they are lower. In this respect, therefore, the two caseins exhibit a slightly different behavior. The divergence is shown further in the values for the electrical conductivity. The two caseins from cow's milk, A and B, furnish digestive products finally which have practically the same conductivity, while from the product from goat's milk, the conductivity is distinctly higher. While in titration this digesting mixture seems to show a low-

er amount of free hydrochloric acid and total acid, it evidently contains groups which furnish a slightly larger number of conducting ions.

It will be noticed further that in all the tests the calculated conductivity, as measured by that of the free hydrochloric acid present according to titration, is always in excess of that actually observed, and that the discrepancy increases as the digestion progresses. It is possible that the "free" acid toward dimethylaminoazobenzene is not all hydrochloric acid as is usually assumed for such mixtures. The lack of sharpness in the indicator may account for some of the discrepancy, but certainly not for all of it. However, as the titrations were always made in the same way, the comparative values remain true. It is also true that the accumulating cleavage products may have some effect in modifying the conductivity of the free acid, but as these products have in themselves some conductivity it is not clear why there should be so great a difference, and in the direction noted, unless we assume that the acid, while apparently free as measured by the indicator, is actually loosely held, and therefore, not really free in the sense of being an ionized conductor.

The control flask referred to at the outset, was used to determine the behavior of the glass in modifying, possibly, the conductivity of the acid. At the end of the series of experiments titration tests showed no appreciable loss of acid by evaporation through the capillary stopper, and the electrical conductivity was practically unaltered. No appreciable solution of the glass had therefore taken place.

Before going into further details of these features of the experiments, it will be well to consider the results obtained with the residues after the completion of the digestions. After making the conductivity tests, the total liquid withdrawn for that purpose, 25 cc., was thrown back into the digestion flask each time. That withdrawn for the titration was, of course, used in such a manner that it could not be returned. The residues were filtered, furnishing the following volumes of liquid, while the solids were collected on weighed paper filters and dried at 100°.

	A	B	C
Volume of filtrate	750 cc.	820 cc.	760 cc.
Weight of residue	0.322	0.342	0.935
Weight for 1000 cc.	0.429	0.417	1.230

These residues, essentially the same for the two lots of cow casein, represent the crude pseudo nuclein from 9.6 grams of casein, as the air dry product used had been found to contain about 4 per cent. of moisture. This gives us, then, as the percentage residues:

A   4.47
B   4.34
C   12.81

It is well-known that the yield of pseudo nuclein is dependent on the

length of the experiment and other conditions as has been pointed out by many observers[1]. The figures show here a marked difference between the two kinds of casein; during the digestion this was evident also in the appearance of the three flocculent residues.

From the clear filtrates 250 cc was evaporated slowly in each case and the residues dried finally at $100°-105°$, leaving perfectly dry, hard, brittle masses of brown color; these were weighed and calculated to a basis of 1000 cc. of filtrate with the results :

A	12.72 grams	
B	12.76	"
C	12.10	"

These numbers point to a great increase in weight by hydrolysis and combination with hydrochloric acid to form complex salts stable at $100°$. The products do not correspond exactly to 10 grams of air dry casein taken, as the 0.5 gram pepsin must also be considered. But as the casein contains about 96 per cent. of dry substance, the weight of 10.1 grams may be considered as the amount hydrolyzed. Making then allowance for the pseudo nuclein filtered off and the pepsin added, the residues just given must be taken as corresponding to the following original weights :

A	9.671 grams	
B	9.683	"
C	8.870	"

The increases in weight, then, are :

A	31.5 per cent	
B	31.8	"
C	36.4	"

These increases are perfectly consistent with the weight of water added in hydrolysis and the combination with hydrochloric acid to form salts or salt-like bodies stable at $100°$. The results show that the goat casein differs materially from the cow casein, the two specimens of which act much alike.

No attempt was made to determine more closely the nature of these residues of digestion. The investigations of Langstein[2], Lawrow[3] and others give some idea of the extent of the cleavage of proteins by the combined action of acid and pepsin. It is likely, as suggested by the researches of Fischer and Abderhalden[4], that numerous products between peptones and the simple amino acids may be included here. Such bodies may resemble the artificial peptides recently studied.

After weighing the dry residues from 250 cc. of digestion liquid they

[1] Sebelien, Z. physiol. Chem. 20, 443.
[2] Beitr. Chem. Phys. und Path., 1, 505.
[3] Z. physiol. Chem. 33, 312, (1901).
[4] See especially, Z. physiol. Chem. 40, 215, (1903).

were redissolved and made up to 250 cc. again. In the evaporation the excess of free hydrochloric acid escaped, and it is likely that further hydrolysis took place. In the new solutions the conductivity at 20° was determined, and also the total acidity with alkali and phenolphthalein, using, as before, 25 cc. of liquid.

	κ_{20}	cc. $\frac{N}{10}$ NaOH
A	0.007725	19.5
B	0.007777	20.0
C	0.007396	18.5

As compared with the figures obtained in the last period of the original digestion, we have here a marked decrease in conductivity, but not as marked a change in the total acidity. The change in conductivity is doubtless due largely to the loss of free hydrochloric acid, while there may have been some gain in the organic acids during the evaporation referred to above; the combined hydrochloric acid held by the various cleavage products must show in the titration. The conductivity of cleavage products of the amino acid type is rather low, but that of their hydrochlorides is much higher[1]. It will be seen, however, that the results are nearly the same for the three caseins if we reduce them to the same basis of the solids present in solution. By dividing the conductivity and titration numbers by the total dry solids present in each case, we obtain the following closely agreeing numbers :

	$10^6 \times \kappa_{20}$ per gm.	cc. $\frac{N}{10}$ NaOH per gm.
A	607	61.3
B	610	62.7
C	.611	61.1

These figures suggest that the products of hydrolysis here obtained must be much alike, whatever differences there may be observed in the extent and character of the digestive operations themselves. The same conclusion must be drawn from recent work of Abderhalden and Schittenhelm[2] on the nature of the two caseins as shown by a comparison of the amino acids obtained by hydrolysis with sulphuric acid.

The results obtained above taken in conjunction with those of a former paper[3], seem to warrant the following conclusions regarding the nature of the two caseins.

1. The caseins from cow's milk and goat's milk are much alike in general behavior. The equivalent weight of the former appears to be slightly lower than that of the latter. Similar salts are formed.

[1] Kanitz, Z. physiol. Chem., **47**, 476.
[2] Z. physiol. Chem., **47**, 458.
[3] Long, this Journal, **28**, 372.

2.   In digestion by pepsin and hydrochloric acid the casein from goat's milk undergoes change more slowly than does the casein from cow's milk and finally from the goat's milk product a larger residue of "pseudo nuclein" is left.

3.   During the digestion the electrical conductivity values and the total and free acidity vary regularly.   Both acidity values are lower for the goat casein than for the other.

4.   After prolonged digestion and separation of the "pseudo nuclein" the residues left contain different weights of amino acids or other cleavage products and their salts.   These weights are much in excess of the "pseudo nuclein"–free casein, and the increase in weight is relatively much greater for the goat casein than for the cow casein.

5.   The conductivity and total acid values of the redissolved solids from the digestion mixtures appear to be much lower for the goat casein than for the other, but if the results are calculated to the basis of unit weight of solids present they are found to be nearly the same, indicating great similarity in the nature of the products formed in the two cases. The most marked difference in the two caseins, therefore, seems to reside in the amounts of the "pseudo nuclein" fraction separable.

Northwestern University, Chicago,
        November, 1906.

THE DETECTION AND ESTIMATION OF α-NAPHTHOQUINONE β-NAPHTHOQUINONE, PHTHALONIC ACID AND PHTHALIC ACID.

BY MAITLAND C. BOSWELL.

During the progress of a research upon the mechanism of the oxidation of naphthalene to phthalic acid, it was found necessary to be provided with methods for the detection and accurate determination of α-naphthoquinone, β-naphthoquinone, phthalonic acid and phthalic acid; and also with a method for the determination of phthalic acid in the mixture of organic acids formed by the oxidation.   As no such methods are described in the chemical literature, I was forced to work them out for myself; and after a number of preliminary trials arrived at the following, which have proved very satisfactory, both as to the simplicity of the operations involved, and the accuracy of the results obtained.

Determination of α-Naphthoquinone :   This compound was prepared by the method of Japp and Miller[1] which is an improvement on the method of Groves[2].

The reaction of the quinone which seemed best suited for the purposes

[1] J. Chem. Soc. **39**, 220 (1881).
[2] Liebig's Ann. **167**, 357, (1873).