Hence we get as conditions for the possibility of the occurrence of barotropic phenomena:

The second component must have: T_{k_2} small compared with T_{k_1}, and by preference also: $M_2 > M_1$ and $b_{22M} < b_{11M}$.

This becomes still clearer by the application of equations (2) and (4) of Comm. N°. 79, April '02, p. 659:

$$x_l = x_g\, e^{\frac{\varphi'_g - \varphi'_l}{MRT}}$$

$$\frac{\varphi'_g - \varphi'_l}{MRT} = \left\{\alpha \frac{T}{p_m}\frac{dp_m}{dT} - \beta\right\}\frac{p_m(v_g - v_l)}{MRT}$$

which determine the ratio of the concentrations of gas and liquid phases of a binary mixture in which the quantity of one component is small, if the law of the corresponding states may be applied. The connodal tangent chord will rapidly deflect from the side $x = 0$, if the exponent of e assumes a considerable negative value. The greatest influence on this exerts $\alpha = \frac{1}{T_{k_1}}\left(\frac{dT_{xk}}{dx}\right)_{x=0}$, on account of the value of the coefficient $\frac{T}{p_m}\frac{dp_m}{dT}$ (> 7); so T_{k_2} will have to be small with respect to T_{k_1}. The influence of $\beta = \alpha - \gamma$, if $\gamma = \frac{1}{v_{k_1}}\frac{dv_{xk}}{dx}$ (cf. Comm. N°. 81, Oct. '02 p. 325) is only of secondary importance. To tend at least in the right direction, γ would have to be negative, so $b_{22M} < b_{11M}$ [1]).

Physiology. — *"An investigation of* Mr. J. W. A. GEWIN, *on the relation of pepsin to chymosin."* By Prof. C. A. PEKELHARING.

That gastric juice possesses the power, on the one hand to digest proteins under acid reaction, on the other hand, to curdle milk under neutral or scarcely acid reaction, is generally attributed to the presence of two different enzymes in the gastric juice, viz. pepsin and chymosin. This opinion is chiefly based upon an observation of HAMMARSTEN, who was the first to throw light on the changes that take place in milk when it is coagulated by means of rennet. HAMMARSTEN found that an extract from the mucous membrane of the stomach, which, when prepared fresh, could digest proteins as well

[1]) The more elaborate mathematical treatment of the conditions for the occurrence of barotropic phenomena, as sequel to Comm. N°. 96*c*, will be postponed till further experiments call for a further discussion.

as curdle milk, after having been digested for a few days with hydrochloric acid at a temperature of 37° C., no longer showed the action of rennet, but had preserved its peptic action. From this it could not but follow that each of these actions depended upon a separate agent.

Meantime doubts have gradually arisen as to the correctness of this opinion. That there must at any rate be a very close connection between the proteolytic action of pepsin and the enzym of rennet, was made probable by the experience that all enzym-solutions with a proteolytic action, no matter whether they are of animal or of vegetable origin, can also act like rennet. And, as I communicated some years ago in this Academy, and as was afterwards corroborated by NENCKI and SIEBER, it could also be proved that all kinds of preparations of pepsin, also when a long digestion with hydrochloric acid and a purification as careful as possible had preceded, are able to act like rennet.

In 1904 there appeared an investigation by PAWLOW and PARASTSCHUK [1]), in which they demonstrated that pepsin and chymosin must be considered as the same substance. These investigators found that in different liquids containing enzym not only the proteolytic and the curdling power are always found side by side, but that also a proportionately greater curdling power corresponds to a greater proteolytic action. That this is not found in some enzym-solutions of commerce appeared to be owing to the presence of other substances; as soon as their effect was destroyed, the proportionality came to light. A solution of rennet, according to HAMMARSTEN prepared by means of carbonate of magnesia from gastric juice, which, in his opinion, no longer contained any pepsin at all, appeared to be a very good digester of albumen, if only the noxious influence of magnesia-salts was taken away. No more was it proved by HAMMARSTEN, — as PAWLOW explained — that a pepsin-solution can be freed from rennet by digestion with hydrochloric acid, as the proteolytic action had been examined, while the liquid still had an acid reaction; the curdling action, on the other hand, after neutralization, by which the enzym might be easily destroged.

Against PAWLOW's explanation objections have been raised. Especially two Swedish investigators, BANG [2]) and SCHMIDT-NIELSEN [3]), have defended HAMMARSTEN's point of view. The investigation of Mr. GEWIN

[1]) Zeitschr. f Physiol. Chemie, Bd. XLII, S. 415.
[2]) Zeitschr f. Physiol Chemie, Bd. XLIII, S. 358.
[3]) Ibid. Bd. XLVIII, S. 92.
[4]) PFLÜGER's Archiv. Bd. LXXIX, S. 425.

refers principally to the grounds alleged by these two authors for the duality-hypothesis.

In the first place he has occupied himself with an inquiry into the correctness of the conclusion previously drawn by BANG [4]) from a number of experiments, that there is not only a difference between pepsin and rennet, but also that even the enzym of rennet does not possess the same qualities in different kinds of animals. BANG continued to apply the old name, chymosin, to the enzym of rennet, as it is found in the calf. From this he distinguished by the name of parachymosin the enzym that can be got from the mucous membrane of the pig-stomach. The difference showed itself in the fact that parachymosin, when diluted, became sooner inactive than chymosin, that it showed a greater activity by the addition of chlorcalcium, was more proof against heating to 70° C. and less so against the action of alkali.

With reference to extracts from the mucous membrane of the stomach of calf or pig GEWIN could corroborate these differences; only he did not find the difference in the promotion of the activity by adding chlorcalcium as important as BANG. However, it was a different thing, if not the extracts themselves were examined but the enzym extracted therefrom by dialysis, and purified as much as possible in the way formerly communicated by me. The better the purification had taken place, the smaller the difference became. The extract from the mucous membrane of the calf-stomach loses its power to curdle milk when, neutralized, it is heated for 10 minutes to 70° C.; however, its power is but little reduced, if it is mixed with caustic soda to 0.01 % and neutralized again after half an hour. With the extract of the mucous membrane of a pig-stomach it is just the reverse. With the enzym of the calf, purified as much as possible, the resistance against heating appeared to have become great, against alkali small. From this it must therefore be deduced that the difference does not lie in the enzym itself, but that it is caused by other substances occurring in the extract. Indeed, it could be proved experimentally that the extract from the membrane of the calf-stomach contains substances which protect the enzym against the action of alkali, but make it the more sensitive to heat. Of a solution of purified pig-enzym (which possesses the qualities of BANG's parachymosin) one half was diluted with water, the other with an extract from the mucous membrane of a calf-stomach, which extract had been deprived of all enzym by heating it for one hour to 80° C. and then neutralized. Of both solutions 2 cc. mixed with 8 cc. of milk caused curdling in 30 sec. A part of each was heated for

10 minutes to 70° C., another part for half an hour left in contact with 0.01 % Na HO and then neutralized again. Now the result was:

	After heating	After action of alkali
2 cc. enzym with water + 8 cc. milk	curdling in 2½ min.	no curdling
2 „ „ „ extract + 8 „ „	no curdling	curdling in 11 min.

GEWIN also examined two rennet-preparations of commerce, one Dutch of VAN HASSELT and one Danish of HANSEN. Both showed the qualities of BANG's chymosin. But when, by dialysis and precipitation with acetic acid, the enzym had been isolated and at least for the greater part been freed from impurities, they had become much more susceptible to alkali and much less so to heating.

That the enzym is destroyed not only by alkaline, but gradually also by neutral reaction has been made clear by PAWLOW and corroborated by GEWIN in numerous experiments. From this GEWIN explains the difference found by BANG between chymosin and parachymosin, the solution being diluted. What BANG calls chymosin is the enzym mixed with substances protecting it from alkali. When those substances are present, it may be assumed that the enzym is better proof against the dilution with water, by which the number of hydroxyl- and metal-ions increases. A solution of purified enzym (parachymosin), possessing the same curdling power as a not purified solution of calf-enzym (chymosin), shows, when diluted, sooner a decrease in action, and consequently must, also sooner, show the promoting influence of the addition of chlorcalcium.

So there is no reason for assuming different rennet-enzymes in different kinds of animals. The difference does not lie in the enzym but in other substances originating from the mucous membrane of the stomach. If it is necessary to give a separate name to the enzym of the gastric juice that can curdle milk, it is sufficient to use the word chymosin for it.

But is even this necessary? Should it be assumed that chymosin is different from pepsin?

To the solution of this question GEWIN has devoted the second part of his investigation. It was tried in vain to divide the enzym into a proteolytic and a curdling part. It is a well-known fact that proteins, undissolved and at a temperature of 15° C. put into a pepsin-solution, take up and keep back this enzym, so that it is not to be separated from it by washing it out. Coagulated and minced hen's albumen was put in a solution of purified pepsin in 0.2 % HCl. Now, if chymosin were a different matter from pepsin, only the last mentioned would perhaps be extracted from the solution by the albumen. It appeared, however, that the liquid filtered from the

albumen after some hours, had lost not only the peptic but also the curdling power. Indeed, the same negative result had already been arrived at by JACOBY, who for these experiments did not use hen's albumen but caseine [1]).

In the second place it was examined whether a separation into two enzymes could be brought about by dialysis. When pepsin, dissolved in hydrochloric acid, is dialyzed against distilled water, it is partly precipitated, most completely at a low temperature, as soon as the quantity of acid has gone down to about 0.02% HCl. Always, however, a considerable part remains dissolved, which, with the aid of ammonium-sulfate — if the solution does not contain much albumose through 50% saturation with this salt — can be precipitated. If pepsin and chymosin are different matters, it cannot be deemed improbable that they also differ in solubility, that therefore the precipitate in the dialyser should contain more of either one or the other matter than the liquid filtered from it. Also in this way, however, a separation into two enzymes, did not succeed.

SCHMIDT—NIELSEN, however, has communicated experiments from which it appears that, though not a complete, still a partial separation of pepsin and chymosin is possible. A strongly active extract from the mucous membrane of the calf-stomach, prepared with hydrochloric acid, was divided into two parts. One was preserved at a low temperature, the other at 37° C. After some days the heated part had for the greater part lost its power to curdle milk, under a neutral reaction; at acid reaction, however, protein was still strongly digested. Now both liquids were neutralized and the one not heated so much diluted that the curdling power had become as weak as that of the one heated. After that the two liquids were rendered equally acid with hydrochloric acid and digested with fibrine. The fibrine was much quicker dissolved by the heated liquid than by the diluted one, not heated. During the process of heating, therefore, the chymosin had been chiefly lost, the pepsin however not.

This experiment would certainly be convincing, if the neutralization had the same effect on the heated liquid as on the one not heated. This, however, is not the case. The extract contains substances protecting the enzym from the action of alkali; also when no more of this is added than what is necessary to attain a neutral reaction. If the extract is preserved at a low temperature, these substances remain for a long time undisturbed, but if the acid extract is heated to 37° C., they are destroyed. GEWIN has proved this by ample experiments, of which a detailed account is given elsewhere. At the

[1]) Biochem. Zeitschr. Bd. I, S. 66.

outset the enzym in the extract of rennet is quite proof against neutralizing, but after having been digested for a few days at 37° C. this power of resistance becomes smaller, and then diminishes quickly. Now, if the neutralized liquid, in order to determine the curdling power, is mixed with milk, the reaction remains neutral and no curdling arises. However, if it is rendered acid again, soon after the neutralisation, a sufficient quantity of enzym is left to digest protein. Also if the not heated solution of the enzym, before being neutralized, is sufficiently diluted with 0.2% HCl, neutralisation herein causes a rapid decrease and at last an annihilating of the curdling power. Thus the curdling time of such a solution was, 8 times diluted, 10 seconds, directly after the neutralisation $2^1/_2$ to 4 minutes, whilst the milk, mixed in the same proportion with the solution half an hour after the neutralisation, was not yet curdled after 20 minutes.

In all experiments, on the other hand, at which the noxious action of alkali was avoided, the curdling and proteolytic power of the enzym solutions appeared to keep pace with each other.

Summing up the result is therefore that not a single reason is left to assume a difference between pepsin and chymosin.

No more is there any reason to stick to the opinion of NENCKI and SIEBER, which I formerly shared, according to which pepsin should be considered as a molecule, which, through different groups of atoms, on the one hand should have a proteolytic, on the other hand a curdling action. The basis for such an idea, the opinion that the activity in one direction could be preserved, whilst that in the other direction was lost, I must now consider as having lost its foundation. The opinion defended by SAWJALOFF, is far more acceptable, who considers the alteration of caseine, of which the formation of cheese is the consequence, as the beginning of digestion, of proteolysis [2]). This opinion has, I believe, become more probable by the experiments made of late about the alterations caseine undergoes under the influence of rennet, particularly by the investigation made some time ago in my laboratory by Miss VAN HERWERDEN [1]). From these it has appeared that caseine, at a very weak acid or neutral reaction and at a temperature not much lower than 37° C. in a solution of rennet — either a preparation of commerce or pepsin purified as well as possible — soon falls asunder into paracaseine, which when it does not directly become insoluble as a lime-compound, cheese, continues to change, and other substances, among which

[1]) Zeitschr. f. Physiol. Chem. Bd. XLVI, S. 307.

[2]) Ibid, Bd. LII, S. 184.

a protein, provisionally called substance C by Miss VAN HERWERDEN. Not until the enzym has been able for a long time to influence these substances, does it form primary albumose from them. At the same time, however, the enzym also appeared at neutral reaction to form from coagulated albumen primary albumose, though in a small quantity. So there is every reason to consider curdling of milk as a proof of the first stage of proteolysis.

Taking this into consideration, it is not so wonderful, as it has been regarded, that all kinds of proteolytic enzymes possess the power of curdling milk, though in natural circumstances they never come in contact with caseine. For then the peculiarity is not to be sought for in the enzyme, but in the caseine, the splitting of which already can be observed in a stage of the digestion, in which with other proteins alteration is still quite imperceptible.

Physics. — "*The intensities of the components of spectral lines divided by magnetism*". By Prof. P. ZEEMAN.

If a spectral line is resolved into a triplet by the application of a magnetic field, the two outer components and the middle line will generally differ in intensity. According to the elementary theory of LORENTZ of the phenomenon of magnetic resolution there exists a simple relation between these intensities.

Let I_1 and I_3 be the intensities of the outer components and I_2 that of the middle line then we may expect that

$$I_1 = I_3 = \tfrac{1}{2} I_2 \quad , \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

It has been often asserted, that generally this relation is not fulfilled, and that triplets frequently have in contradiction with (1) a weak middle line and strong outer components.

Really some cases [1]) can be cited, in which the intensities differ from what may be inferred from equation (1). In numerous cases however this contradiction is only apparent, no attention having been paid to a circumstance presently to be mentioned and not yet examined in connexion with our present subject.

In the very important investigation by RUNGE and PASCHEN [2]) a calcspar prism was placed before the tube placed in the magnetic field. By means of a quartz lens the two images given by the calc-

[1]) The lines exhibiting the partial polarization observed by EGOROFF and GEORGIEWSKY (C.R. **124**, **125** 1897) are meant here.

[2]) C. RUNGE u. F. PASCHEN Abh. der Berl. Akad. Anhang. 1902.