ing values $t_1, t_2, \ldots t_p$ of t, then $3n+p-2-\sum_{i=1}^{p} k_i$ will indicate the class of R_h.

4. In the preceding number we have dealt with the class of R_h only, without taking the other characteristic numbers into consideration. We now immediately add that the rule according to which the envelope of a space with $s-1$ dimensions, the equation of which contains a parameter to degree k, is characterized by the numbers

$$k, \qquad 2(k-1), \qquad 3(k-2), \quad \ldots\ldots \quad s(k-s+1)$$

in general needs some modifications as soon as one of the above-mentioned particular cases appears. In the very simplest case of the parabola we find e. g. for the characteristic numbers, class and order, of the evolute 3 and 3, but not 3 and 4 as might be expected for $k=3$. So in general in each of the particular cases treated here the numbers k, $2(k-1)$, $3(k-2)$, etc. must be treated as upper limits.

In a following paper we shall revert to this last point.

Physiology. — "*Lipolytic ferment in ascites-liquid of man*". (Remarks on the resorption of fat and on the lipolytic function of the blood). By Dr. H. J. HAMBURGER.

(Read January 27, 1900.)

In an essay published in the year 1880 CASH [1]) has contradicted the opinion that the emulsion of fat already takes place in the intestinal lumen. For he was never successful in separating an emulsion from the contents of the intestines by centrifugal force. And he did not much wonder at this: for the small intestine has an acid reaction, and with acid reaction no fat-emulsion can be produced.

This opinion of CASH does not seem quite correct to me. Giving to animals a meal containing much fat, HEIDENHAIN has found [2]), and so have I myself many a time, that a creamy surface can be taken off the mucosa of the small intestine, which, examined microscopically, contains small fat-globules. Nevertheless this layer

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1) Archiv f. Physiol. 1880. S. 323.

2) PFLÜGER's Archiv. 1888, supplement, S. 93.

has an- acid reaction. That acid reaction can be coexistent with exquisite emulsions, has been proved by J. MUNK, who obtained emulsions by mixing pure fatty acid with a little $Na_2 CO_3$-solution. Another question is however whether the emulsion is already found so finely divided in the intestinal lumen as later on in the chyle-vessels. This now is certainly not the case. Even in the epithelium-cells and in the adenoid tissue of the villi relatively large globules of fat are found, and it is only in the chyle that it appears in its peculiar dust-shape.

It can scarcely be doubted that in the lymph of the villi a cause must exist which brings about the transition of fat to the form of dust.

In order to test this supposition it would be well to gather chyle, undo it by means of a CHAMBERLAND's candle of particles of fat and afterwards shake the clear liquid with fat. It is however scarcely possible to obtain the necessary quantities of chyle for the purpose.

I happened to learn that in the Hospital of the Utrecht University a patient was treated, whose abdomen contained a large quantity of ascites-liquid, which had the appearance of chyle. Professor TALMA kindly put it at my disposal.

Upon close microscopical investigation however the liquid showed not a single particle of fat and it soon appeared that the observed opalescence proceeded from a mucoid substance which was first described by HAMMARSTEN [1]) and the existence of which was later on confirmed by different medical men [2]).

As regards its composition, the liquid contained 1.939 pCt. solids, consequently less than normal lymph; in which, as is well known, circa 4 pCt. solids are present. It contained 1.715 pCt. albumen, 0.0808 pCt fat, and 0.0564 pCt. soap.

The extraordinary insignificant quantity of fat proved that this was not a case of real chylous ascites, as one could have believed at first sight.

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[1]) O. HAMMARSTEN. Ueber das Vorkommen van Mucoidsubstanzen in Ascitesflussigkeiten. Autoreferat in Maly's Jahresber f. Thierchemie, uber das Jahr 1890. S. 417.

[2]) S. und A. L. PAYKUL. Beitrage zur Kenntniss der Chemie der serosen Exsudate. Ref. Jahresber. f Thierchemie, uber das Jahr 1892. S. 558.

G. LION. Communication d'un cas d'ascite laiteuse ou chyleuse. Arch. de méd. expériment. 1894, p. 826.

OFCONI, Ueber einen Fall milchig getrubten nicht fetthaltigen Ascites. Italiaansch in Ritorma mediche, 1897, no 51. Ref. Maly's Jahresber. f. Thierchemie, uber das Jahr 1897. S. 190.

It was proved by laparatomy that the patient was suffering from cirrhosis hepatis and slight chronic peritonitis.

Although the liquid was not chylous, we have nevertheless examined it in the proposed direction, because lymph from other parts of the body seems likewise to have the property to divide fat into the smallest grains. Think of GIMBERT's [1]) experiments; he repeatedly injected into the human body, not only without harm, but with favourable influence on the general condition 25—30 gr. olive—oil with 1 : 15 creosote. The experiments of LEUBE should also be remembered [2]). He was encouraged by the experience made with respect the human body, that subcutaneous injections of camphorated oil applied even in large quantities, can be borne without disad vantage, and consequently tried subcutaneous injections of fat on dogs and thus obtained a considerable deposit of fat in different parts of the body.

Finally I quote the experiments of J L. PRÉVOST [3]) according to which the oil injected into the lymphbag of frogs, appears as tiny globules in the circulation.

It must be taken for granted that the fat can undergo a minute division in the tissue spaces; otherwise mortal emboli, for instance in the lung-capillaries, would undoubtedly have followed these experiments. With regard to this it is interesting on the other hand that DAREMBERG [4]) by subcutaneous injections on rabbits and Guinea pigs, caused death.

Furthermore 50 cc. of the ascites-liquid with 5 cc. of lipanine were shaken together. In this manner an emulsion was formed, which, by standing motionless and also by centrifugalizing separated itself into two layers. The upper layer examined microscopically, showed large fatglobules; the lower one, particles as tiny as dust, similar to those that are found in chyle and also in milk, the cream having been taken off by centrifugalizing. After that the lower layer was removed and once more centrifugalized. It remained however equally untransparent.

Why had the emulsion separated itself into two layers?

Is it because the oil contained two different kinds of fat, of which the one gives an emulsion *as fine as dust*, but not the other

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[1]) Compt. rend. de la Soc. de Biol. T. 40, 1889, p. 733.

[2]) Sitzungsber. der physik. med. Gesellsch. zu Wurzburg. 1895. S. 1 no. 5.

[3]) Travaux du laboratoire de thérap. expérim. de l'Univers. de Genève, II. 1896, p. 44.

[4]) Compt. rend. de la Soc. de Biol. T.40, 1889, p. 702.

one? Or were the conditions not favourable for a thorough dust-emulsion of the *whole* mass of fat?

In order to decide this question the uppermost layer (large drops of fat) was taken off by means of a pipette and shaken anew with fresh ascites-liquid. Centrifugal force was again applied and once more a separation into two layers was visible. Both layers contained fat; now the undermost layer even contained more fat than at the beginning of the experiment and the microscope only showed the dustshape. From this it was evident, that the part of the fat, which, with the first experiment was separated in the shape of globules into an uppermost layer, had been transformed into fat in the shape of dust by shaking with fresh ascites-liquid.

That which had not passed into dust was shaken again with fresh ascites-liquid, and now at length all the fat had been turned into the shape of dust.

That with the first shaking-experiment the fat only partly passed into dustform, does not find its cause in an eventual difference in the relative condition of the different kinds of fat in the oil, but can be explained from the conditions of the experiment. It has indeed been proved that a perfect dust-shaped emulsion can be obtained *at once*, if only the shaking is continued for a long time and with a relatively large quantity of ascitesliquid.

I have further considered whether a peculiar quality of the ascites-liquid must account for this. Therefore the experiment was repeated with another albuminous liquid, viz. with bloodserum, 30 cc. horse-serum were mixed with 5 cc. lipanine and the mixture strongly shaken for one hour. The emulsion was next centrifugalized, and thereby divided itself into two layers, a lower one with fat in dust-shape, an upper one with tiny fatglobules.

The latter was removed, vigorously shaken with 30 cc. of the fresh serum and after that centrifugalized again; once more two layers were obtained; the lower one however now contained much more fat than with the first shaking. After having been shaken third time with 30 cc. serum, all the fat was brought into the form of dust. Shaking 150 cc. serum with 5 cc. lipanine for four hours, brought about the perfect dust-shaped emulsion. This emulsion could now no more be divided into two layers by centrifugalizing. We thus did not find any specific quality in our ascites-liquid with regard to the dispersion of the fat, for the same occurred with the blood-serum.

Transferring these facts to normal life — which does not seem too hazardous in this case — it can be imagined, that the lymph

of the villi in its *motion*, causes the little fatglobules, already in a state of thorough division, to pass into the shape of dust. Surely this lymph-current works slowly, but it should be considered, that the time at its disposal is not short; 30 hours after a rich meal has been taken the chyle still carries away fat.

As is well known COHNSTEIN and MICHAËLIS have pointed out in two interesting publications [1]), that, when blood has been mixed with chyle-fat and air is then carried through the mixture, the fat disappears, and a combination dissoluble in water takes its place. We were interested to know, when blood is mixed with our artificial chyle (dust-shaped emulsion of lipanine in ascites-liquid) and a current of air was made to pass through, whether a disappearance of fat would likewise be observed.

To this purpose 240 cc. of the ascites-liquid with 15 cc. lipanine were shaken for $1^1/_2$ hour. After centrifugalizing, the undermost of the two layers is removed, which contains the fat exclusively in dust-form.

Of the artificial chyle attained in this manner:

(1) 75 cc. was mixed with 25 cc. horse-blood rich in erythrocytes [2]). For 23 hours a current of air is allowed to pass through under a temperature of ± 16° C.

(2) 75 cc. of the artifical chyle are mixed with 25 cc. blood. *No* current of air is allowed to pass through.

The liquids (2) are mixed just before drying takes place.

At the same time exactly the same experiments are performed with dust-shaped lipanine-*serum*-emulsion, consequently:

(3) 75 cc. of a dust-shaped lipanine-serum-emulsion are mixed with 25 cc. horse-blood, and through this mixture air is allowed to pass through for 23 hours (the same current of air as under (1).

(4) 75 cc. of the dust-shaped lipanine-serum-emulsion are mixed with 25 cc. of blood. *No* current of air passes through.

The liquids (4) are mixed just before drying takes place.

(1), (2), (3) and (4) are placed into two small receptacles, mixed with 20 gr. of pure sand and being stirred, dried in a temperature of 80°. After having been pulveriged, extraction with ether, free of water, in a Soxhlet apparatus for 48 hours.

From (1) . . . . . 0,244 gr. ether residu
" (2) . . . . . 0,475 gr. " "
were be obtained.

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[1]) Sitzungsber. der Preussischen Akademie der Wissensch. 1896. S. 171; more circumstantially in PFLÜGER's Archiv. B. 65, 1897 S. 76; B. 69, 1897, S. 473.

[2]) Such blood is obtained by leaving defibrinated horse-blood to itself and by pipetting off the serum after the red blood-corpuscles have settled down.

By this we have proved, that by letting the air stream through the mixture of blood and lipanine-ascites-emulsion a considerable transformation of fat takes place.

From (3) 0.371 gr. Ether residu is obtained
" (4) 0 283 gr. " " " "

From these two numbers it is evident that by letting a current of air pass through a mixture of blood and *dust-shaped* lipanine-*serum*-emulsion, no transformation of fat takes place.

These numbers even rather tend in the opposite direction.

After all these experiments it must be taken for granted, *that the lipolytic ferment was not present in the blood, nor in the serum, but existed in the ascites-liquid.*

The question could now be examined, whether the presence of blood is really required for the transformation of fat and whether it is not sufficient to pass air through the lipanine-ascites-emulsion.

To reply to this question, 80 cc. of a lipanine-ascites-emulsion (75 cc. ascites-liquid + 5 cc. lipanine) were shaken for 3 hours and submitted to a current of air for 20 hours. After that the fat-contents were determined, which took place at the same time with a portion of the same emulsion, not having been treated with a current of air.

80 cc. lipanine ascites-emulsion treated with air contained 4.300 gr. of fat
80 cc. " " " not " " " " 4.252 " " "

The passing through of air only, has consequently not given cause to transformation of fat.

This result agrees with that of COHNSTEIN and MICHAËLIS. These investigators also found in their experiments with true chyle, that without the presence of red blood-corpuscles, the passing through of a current of air was not able to cause a transformation of fat.

Repetition of the experiment.

This experiment was performed in the same manner as the foregoing; the only difference being that instead of 24 hours, the current of air was only allowed to pass for $12^1/_2$ hours, under room-temperature, and instead of horse-blood, ox-blood was used.

(1) 75 cc. of dust-shaped lipanine ascites-emulsion + 25 cc. ox-blood. Current of air for $12^1/_2$ hours; after that the liquid is mixed with sand, dried an extracted with ether. Ether-extract 0.064 gr.

(2) 75 cc. of the dust-shaped lipanine-ascites emulsion are mixed with 25 cc. ox-blood, although not before a current of air has been led through the mixture (1) during $12^1/_2$ hours. After intermixing, the liquid is treated at the same time and in the same way as in (1). Only here, as has been mentioned, no air is allowed to pass through. Ether-extract 0 186 gr.

(3) 75 cc. of the dust-shaped lipanine-ascites-emulsion produce an ether-extract of 0.219 gr.

(4) 75 cc. of dust-shaped lipanine-*serum*-emulsion are mixed with 25 cc. ox-blood. Current of air for $12^1/_2$ hours. Dried with sand, extracted with ether. Ether-extract 0.359 gr.

(5) The same as experiment (4), however without the current of air. Ether-extract 0.364 gr.

(6) 75 cc. of the dust-shaped lipanine-serum-emulsion. Ether-extract 0.369 gr.

It appears from (1) and (2), that in passing air through the mixture of blood and dust-shaped lipanine-ascites-emulsion (artifical chyle), fat disappears.

On comparing (2) with (3) it appears that also when a current of air is not passed through, a little fat is analysed. As the experiments of COHNSTEIN and MICHAËLIS have pointed out, and we have been able to confirm, this transformation takes place in consequence of the drying of the emulsion in presence of blood and air.

It appears from (4) and (5), that the passage of air through the mixture of dust-shaped lipanine-*serum*-emulsion and blood, causes *no* transformation of fat, which is confirmed by the result of (6).

Two repetitions of the experiment.

Ox-blood was now again taken; duration of the passage of air 28 and 18 hours. Room-temperature.

(1) 75 cc. dust-shaped lipanine-ascites-emulsion + 25 cc ox-blood. Passage of a current of air through the mixture for 18 hours. After that dried with sand and extracted with ether. Ether-extract in the two experiments 0.215 Gr. and 0.114 Gr.

(2) 75 cc. of the dust-shaped lipanine-ascites-emulsion are mixed with 25 cc. ox-blood, after air has been conducted through the former mixture for 18 hours; after mixture the whole mass is treated instantaneously, consequently at the same time with (1), for the determination of fat. This experiment is therefore similar to (1); with the exception that no air is conducted through. Ether-extract 0.498 and 0.288 Gr.

(3) 75 cc. of the dust-shaped lipanine-ascites-emulsion give from 0.562 and 0.315 Gr.

(4) 75 cc. of the dust-shaped lipanine-serum-emulsion are mixed with 25 cc. oxen-blood. Passage of air for 18 hours. Drying with sand, extraction by means of ether, free of water. Ether-extract 0.401 and 0.312 Gr.

(5) Same experiment (4), but without passage of air. Ether-extract 0.394 and 0.321 Gr.

(6) Passage of air through 75 cc. of the dust-shaped lipanine-ascites-emulsion. Ether-extract 0.567 Gr.

On comparing (1) and (2) it appears again, that by passing air through the mixture of blood and dust-shaped lipanine-ascites-emulsion, disappearance of fat takes place.

On comparing (2) and (3) it appears that by non-conduction of air, some fat is transformed as well. This transformation occurs whilst the drying is going on, as long as the temperature still remains below the transformation-temperature of the ferment.

(4) and (5) show, that conduction of air through the mixture of blood and dust-shaped lipanine-*serum*-emulsion, causes *no* transformation of fat, which is confirmed by the results drawn from (6).

Finally the comparison of (6) and (3) proves, that without the aid of blood, the passing through of air is not efficient to make the fat disappear. Considering the results of the different experiments, there is no doubt, that in the examined ascites-liquid a substance exists which appears able to transform fat, and which, with the aid of bloodcorpuscles and with access of oxygen, performs the change.

COHNSTEIN and MICHAËLIS are of opinion that this substance, with which they obtained such a transformation of the chyle-fat, is contained in the blood which was used by them.

Closely considering their experiments it strikes us that they have no right to maintain this conclusion. For when they observe that after mixing blood with chyle, *fat* disappears from the latter, it is notwithstanding possible, that the ferment is not present in the blood, but in the chyle. It must seem strange that the authors have not considered this possibility, because *no* fat disappeared from the mixtures of *milk* and blood and from *codliver-oil*-emulsions with blood. The authors have tried to explain this latter fact by taking for granted, that the fat would be present in the chyle in a more finely divided condition. Meanwhile this explanation does not seem satisfactory to the investigators themselves, and it cannot be correct, for as was mentioned above, fat also appears in milk in dust-shape. The fat of the so-called undermilk (the undermost of the two layers in which the milk is separated when contrifugalized), consists exclusively of dust-particles; it amounts to about $^1/_{20}$ of the total quantity of fat.

Also from emulsions of codliver-oil with $Na_2 CO_3$, a portion can always, by centrifugalizing, be separated as emulsion in dust shape.

It would perhaps be possible — also in connection with what we found in our ascites-liquid — to find the explanation of their negative result with milk and codliver-oil, in the fact, that neither in milk and codliver-oil, nor in blood a lipolytic ferment was present,

but that it was present in the chyle; hence the transformation of fat in a mixture of blood and chyle.

To my regret I was obliged to cut short my investigation on this subject Although I am fully aware that these results are incomplete in many ways, it seemed expedient to me to publish them at present, as for some time I shall not have the opportunity to pursue this subject, and I wished to stir up other inquirers to the use of ascites-liquid for the study of the lipolytic ferment. The mucoid ascites-liquid can be had in such abundance (repeatedly more than 8 Liter of liquid were removed from the abdominal cavity of the patient), that it will afford a better and more extensive opportunity for the study of the nature and the effects of the lipolytic ferment, than most other animal ferments.

The above mentioned researches have given the following results:

1. It is possible to make from lipanine (acid olive oil) a perfect dust-shaped emulsion. This has not only been successfully performed with the aid of the examined mucoid ascites-liquid, but also with ordinary horse-blood-serum.

2. This fact seems to indicate, that during life, the transition into dust-shape of the small fat globules, which still exist in the adenoid tissue of the villi, is caused by the continuous motion of the lymph of the villi.

3. The opalescent, non-fatcontaining, mucoid ascites-liquid examined by us, contains a lipolytic ferment, which possesses the power to transform dust-shaped fat. For this transformation the presence of bloodcorpuscles and also access of air is necessary.

4. The contention of COHNSTEIN and MICHAËLIS, that the lipolytic ferment discovered by them, originates from the blood, has not been proved. Their and my experiments rather show, that the ferment is a constituent of the chyle.