Biochemistry. — "*The antagonism between citrates and calciumsalts in milkcurdling by rennet. A contribution to the knowledge of the relation between structure and biological action*". (First communication). By J. R. KATZ. (Communicated by Prof. A. F. HOLLEMAN).

(Communicated in the meeting of April 26, 1912).

Introduction.

A small quantity of neutral citrates prevents the coagulation of blood; various salts which precipitate calcium as an insoluble compound, e.g. oxalates and fluorides, have an analogous action. The peculiarity of the case is however that citrates do not precipitate diluted solutions of calciumsalts, but remain entirely clear; notwithstanding this, they have neutralized the effect of the calcium.

A similar antagonism between citrates and calcium has been found in various biochemical and pharmacological processes.

Citrates in small quantity prevent the curdling of milk by rennet; various immunochemical reactions are prevented by citrates, as the laking of red bloodcorpuscles by animal hemolysines (eelserum cobralecithide, normal complement); here also the action of citrates is prevented by the addition of calciumsalt [1]).

Recently Prof. HAMBURGER has shown that the phagocytic power of the leucocytes is inhibited by citrates and is reactivated by calciumsalts and that fluorides and oxalates too prevent phagocytosis.

The pharmacological action of citrates shows the same antagonism with calciumsalts, e.g. JANUSHKE [2]) has found that the paralysis of the heart and the paralysis of the nervous system caused by intravenous injection of citrates, is removed by injection of calcium. BUSQ and PACHON [3]) showed, that there exists antagonism between the action of citrates and calcium on the heartmuscle, and MAC CALLUM [4]) observed that the purgative action of citrates is inhibited by addition of calciumsalt.

It seems perfectly clear, that substances as fluorides and oxalates which precipitate calcium as a nearly insoluble compound, are antagonists of calcium, but how shall we explain, that citrates have the same action, although they do not precipitate calciumsalt?

As this property of citrates is used more and more in hema-

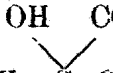
[1]) GENGOU, Arch. Intern. de Physiologie 7 (1903).
[2]) Arch. f. Exper. Pathol. u. Pharmak. 61. p. 363—375.
[3]) C. R. 148 p. 575—578.
[4]) Americ. Journ. of Physiol. 10, p. 101—110.

tological and immunochemical reactions, it has drawn attention from various sides. SABBATANI [1]) thinks, that its action might be caused by the diminution of the number of free calciumions; ARTHUS [2]) and GENGOU [3]) showed, that citrates have an antiflocculant action on finely divided suspensions, and asked whether the biological action should not rather be attributed to the latter. Finally M. H. FISCHER [4]) proved recently that citrates inhibit to a large extent the swelling (imbibition) of proteids by acids and by alkalis and that some pharmacological properties of these salts (e.g. their influence on glaucoma) are related to this. Each of these theories is supported by a number of experiments; possibly all three contain a part of the truth; under these circumstances a choice between the different explanations does not seem possible as long as the biological action of citrates has not been more extensively analysed.

Analysis of the biological action of citrates by comparison with the action of substituted citrates.

For such an analysis the finding of the active groups in the citrate-molecule seems particularly adapted. For it seems rather improbable that the different actions of the citrates are all caused by the same groups. Then this research will give an indication which actions can be compared, which not.

In order to find which are these groups, I have followed the ordinary pharmacological method. A number of derivatives of citric acid were prepared in which the probably active groups were changed in different ways. As all acids which precipitate calcium in the manner of fluorides and oxalates inhibit the curdling of milk, this secondary complication should be excluded. On this account I have made a control with all acids examined in order to see if the solution of the neutral salt of the acid used, precipitated a diluted solution of calciumsalt. Only those, which did not were used for the investigation. As solution of calciumsalt I chose a solution of gypsum (saturated solution, diluted 1 : 5 with distilled water).



Citric acid, $COOH . CH_2 . C(OH)(COOH) . CH_2 . COOH$, contains 4 groups which may be considered as the active ones: three carboxylgroups COOH and one alcoholgroup OH. The hydroxylgroup can be made inactive

[1]) Atti della R. Acad. di Torino **36**, p. 27—53; Memorie [2] **52**, p. 213—257.
[2]) C. R. Soc. de Biol. **36** (1901).
[3]) l. c.
[4]) Das Oedem.

by acetylation; of the carboxylgroups one, two, or three can be removed by preparing the mono-, di- and tri-amides, or mono-, di- and tri-esters [1]).

a. The alcoholgroup made inactive.

The acetylcitric acid $COOH . CH_2—\overset{C_2H_3O_2\ COOH}{\overset{\diagdown\ \diagup}{C}}—CH_2 . COOH$

Anhydrous citric acid was boiled with acetylchloride according to EASTERFELD and SELL [2]); the acetylcitric anhydride formed is purified by washing with acetylchloride and dried in the exsiccator above sodium hydroxide. Immediately before use, this substance was recrystallised with chloroform until it had the exact melting-point. A weighed quantity of this substance was dissolved in water of 50° C., when the anhydride changes into acetylcitric acid, and then was neutralized with titrated sodium hydroxide solution. Three equivalents of sodium hydroxide were used pro molecule acetyl-citric anhydride, as should be the case when no acetic acid is split off. Such a solution is relatively very stable at ordinary temperature and only becomes acid after several weeks (by breaking up into acetic acid and citric acid).

When the alcohol-group of citric acid is made inactive, the substance has become comparable with other multi-basic aliphatical acids without alcohol group. It therefore is interesting to compare some of these acids with acetylcitric acid as to their action on milk-curdling [3]).

For comparison were chosen:

aconitic acid $COOH . CH_2 \overset{COOH}{\overset{|}{C}} = CH . COOH$

Purity controlled by melting point.

isoallylentetracarbonic acid $COOH . CH_2 . \overset{COOH\quad COOH}{\overset{\diagdown\ \diagup}{C}} . CH_2COOH.$

The tetra-ethylester of this acid was prepared by condensation of malonic ester with 2 molecules of monochlor-acetic-

[1]) I am indebted to Dr. J. BLANKSMA for his amiable advice in synthetical difficulties.

[2]) Journ. of the Chem. Soc. **61**, p. 1003—1012.

[3]) In order to prevent secondary complications I have for comparison chosen acids which differ as little as possible in structure from the original ones Acids in which the carboxylgroups are nearer to one another than in the citric acid-molecule were excluded, because in such cases other properties so often appear.

acid ester. This tetra-ethylester was saponified in alcoholic solution according to the method of BISCHOFF [1]; addition of $BaCl_2$ precipitated the bariumsalt. This starchy looking salt could not be sucked dry; it was purified by repeated decantation. The free acid was prepared by adding the calculated quantity of sulphuric acid; it was extracted with ether and recrystallised with anhydrous ether until it had the right melting-point (this never was quite exact because of the decomposition on melting).

tricarballylic acid $COOH . CH_2 . \underset{\displaystyle H \quad COOH}{C} . CH_2 . COOH$

b. one carboxylgroup made inactive.

Symmetrical citric monoamide $COOH . CH_2 . \underset{\displaystyle OH \quad CONH_2}{C} . CH_2 . COOH.$

To pure acetondicabonicacid-diethylester prussic acid was added in statu nascendi; the cyanhydrine was saponified with strong sulphuric-acid, after dilution of the H_2SO_4 with ice the diethylester of monoamide-citric acid was extracted with chloroform. This was purified by pressing between unglazed porcelain plates, dissolving in chloroform and precipitating with ligroine, until it had the required melting point and was colourless.

To a weighed quantity of this substance a small excess of normal sodium hydroxide solution was added; after 24 hours only the 2 estergroups were saponified as was proved by titration. The amide is very resistant to diluted solutions of sodium hydroxide at ordinary temperature [2].

Such a substituted citrate, in which one carboxylgroup has been made inactive, was compared with some acids with two carboxylgroups and one or more hydroxylgroups. As such were chosen:

the *malic acid* $COOH . \underset{\displaystyle OH}{CH} . CH_2 . COOH$

the *tartaric acid* $COOH . \underset{\displaystyle OH}{CH} . \underset{\displaystyle OH}{CH} . COOH$

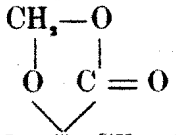
the *trioxyglutaric acid* $COOH . \underset{\displaystyle OH}{CH} . \underset{\displaystyle OH}{CH} . \underset{\displaystyle OH}{CH} . COOH.$

[1] Lieb. Ann. **214**, p. 61–67.

[2] SCHROETER, Berl. Ber. **38**, p. 3199.

Pure arabinose, prepared from arabinose was oxydised at 35° C for 24 hours with 2½ parts of nitric acid (spec. grav. 1,2). The superfluous HNO_3 was removed in the waterbath and the residue dissolved in 25 parts of water. This liquid was saturated at its boiling temperature with calciumcarbonate and filtrated while hot. The calciumsalt separated on cooling. The potassiumsalt was formed by adding the calculated quantity of potassiumcarbonate, decoloured with animal charcoal and purified by recrystallisation. As the calciumsalt is soluble only to a small extent, the acid could only be used in diluted dilution[1]).

c. The alcoholgroup and one carboxylgroup made inactive.



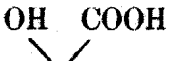
The *methylencitric acid* $COOH.CH_2.C.CH_2.COOH$.

Methylencitric acid is formed by treating citric acid with formaldehyde and separating from the unchanged citric acid. I was presented with this substance in a very pure condition (as neutral sodiumsalt) freshly prepared by the Pharmaceutical Laboratory of the Elberfelder Farbenfabriken (Bayer)[2]).

This compound was compared with some other dibasic acids without alcoholgroup:

succinic acid $COOH.CH_2.CH_2COOH$.
glutaric acid $COOH.CH_2.CH_2.CH_2COOH$
pimilinic acid $COOH(CH_2)_5.COOH$.
suberic acid $COOH.(CH_2)_6.COOH$.

d. two or three carboxylgroups made inactive.



Symmetric citric acid dimethylester $COOCH_3.CH_2.C.CH_2.COOCH_3$

100 gr. of citric acid were dissolved in 500 gr. methylalcohol and boiled for one hour after addition of 4 gr. H_2SO_4; this mixture was diluted with limewater, neutralized with $CaCO_3$ and filtered. The filtrate was concentrated in vacuo. After addition of HCl. the ester crystallized and was recrystallized from water. Melting-point (not very sharp) 125-127° C.[3]).

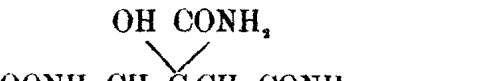
[1]) KILIANI, Berl. Ber. 21, p. 3007.
[2]) I am sincerely indebted to the Elberfelder Farbenfabriken for this kindness.
[3]) SCHROETER Berl. Ber. 35, p. 2086.

Citrodiamide (symmetric?) $CONH_2.CH_2.C.CH_2.CONH_2$.

The motherliquor of the citramide (see below) was acidified with nitric acid and precipitated with alcohol. The citrodiamide is gained as a white crystalline powder [1]). Usually the compound is mixed with its ammoniumsalt.

Citric acid trimethylester . $COOCH_3.CH_2.C.CH_2.COOCH_3$.

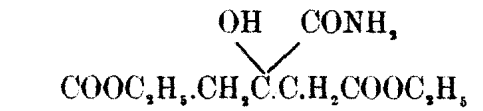
One part of citric acid was dissolved in one part of methyl alcohol and the solution saturated with HCl-gas. On cooling the ester crystallized and was purified by recrystallizing from methylalcohol. Purity controlled by melting-point.



Citramide $COONH_2.CH_2.C.CH_2.CONH_2$

Citric trimethylester was dissolved in 5 parts of strong ammonia (spec. grav. 0.88); soon the citramide precipitated and was recrystallized from water [2]).

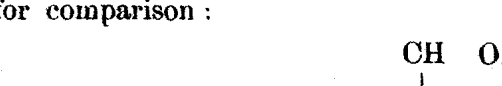
Diethylester of monoamide-citric acid



preparation described on page 437; purity controlled by melting-point.

The action of these substituted citrates was compared with the action of other organic compounds, having none or only one carboxyl group, but one or more hydroxylgroups.

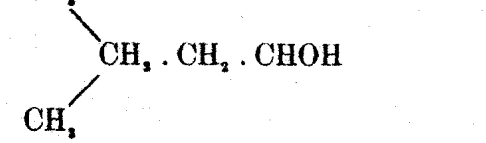
We choose for comparison:



monoethylester of tartaric acid $COOC_2H_5 . CH . CH . COOH$

preparation of MERCK.

isoamylalcohol CH_3.



[1]) When no crystals of this substance are obtainable, it may last months before crystallization begins.

[2]) BEHRMANN und HOFMANN, Berl. Ber. 17, p. 2684.

glycerine	$CH_2OH . CHOH . CH_2OH$
erythrite	$CH_2OH . CHOH . CHOH . CH_2OH$
mannite	$CH_2OH . (CHOH)_4 . CH_2OH$
glucose	$CH_2OH . (CHOH)_4 . COH$

Influence of substituted citrates on curdling by rennet.

It seemed natural to begin the investigation with that biochemical or pharmacological process, the nature of which seemed most simple and by which the smallest number of complicating circumstances might be expected.

To begin with, pharmacological actions may be excluded. For, an intravenously injected substance only acts after having been taken up by the tissues; the really acting concentration therefore is not only determined by the injected quantity (calculated pro kilogram of bodyweight) but also by the partition-coëfficient tissue-blood, which is very different for different substances.

The same difficulty complicates the investigation of immunity reactions; here also the partition-coefficient leucocytes-serum or erythrocytes-serum varies considerably for various substances.

Remains the coagulation of blood and milk-curdling by rennet. Milkcurdling seems to be a so much simpler process than bloodcurdling, the substances one has to work with, so much more stable, that milkcurdling seems to be the natural process to begin with. It is my intention to study later immunochemical and pharmacological processes with this method.

In order to find the influence of citrates on the curdling of milk, I first observed how much the curdling-time was lengthened after addition of increased quantities of neutral citrate of sodium. I prepared a $^1/_{40}$ normal solution of sodiumcitrate, to which 2 drops of litmustincture were added and which by addition of a few drops of dilute hydrochloric acid or sodium hydroxide were brought to the same colour as distilled water with the same quantity of this indicator. 1 40 normal solution obtained in this way, was diluted to an 1/80 N, 1/100 N, 1/200 N, 1/500 N and 1/1000 N. I convinced myself that all these solutions remained neutral.

In order to determine the curdling-time, 10 cc. of raw milk were pipetted into a test-tube, 2 cc. of distilled water, resp. a solution of citrate of different strength, were added, the test-tube was closed with a cork or a stopper of cotton-wool, well mixed and placed in a waterbath of 37° C. until it had reached this temperature. Then 0.5 cc. of a solution of commercial rennet in distilled water (1 : 17)

was added with a pipet, the contents of the test-tube quickly reversed several times and again put into the waterbath. By carefully moving the test-tube from time to time, the moment when the milk became thicker, could be observed. When this change began, curdling was very near. The test-tube then was taken out of the waterbath and carefully inclined, so that some of the contents slowly moved along its walls, till at a certain moment floccules of about $^1/_4$ m.m. suddenly appeared in the milk which adhered at its walls. This point was taken as the curdling-point. As milk-curdling is delayed by shaking and by cooling, care was taken to avoid all unnecessary movement and cooling. With some practise it is easy to reduce both factors to a minimum and then the curdling-time can be accurately determined. In the case of milk without citrate, the curdling-time seldom varied more than 15 seconds (in a curdling-time of $2^1/_4$ minute); usually the observations differed less. After adding salts which only give a small delay, analogous differences were obtained; in the case of strongly delaying salts, the differences were somewhat larger, but always agreed sufficiently. Every curdling-time was determined in duplo or in triplo and the exact values found by taking the average. Curdling-times of more than 2 hours cannot be trusted because of the possibility of bacterial action.

It was found, that the kind of milk investigated on subsequent days with the same solution of rennet (1 : 17) gave curdling-times which varied little. In order to make observations on different days as well comparable as possible, the solution of rennet was always taken somewhat more or less diluted till a curdling-time of 2 minutes 18 seconds exactly was obtained, this being the value on the first day.

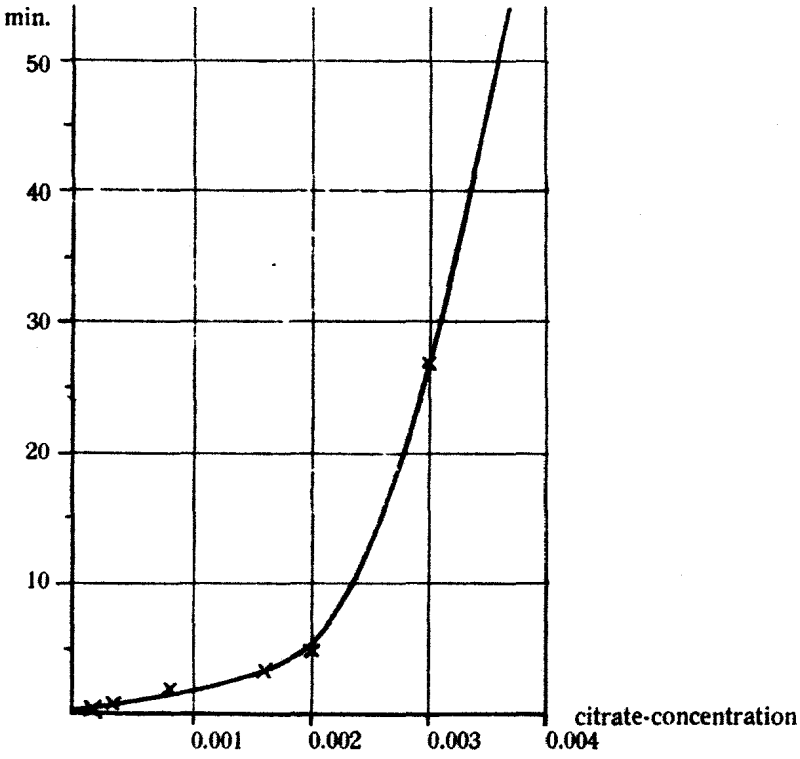
The lengthening of curdling-time found when the milk contained the quantities of citrate given below, is seen from the following figures:

0.00016 N	delay	17	seconds	0.0020 N	delay	289	seconds
0.00032 N	,,	34	,,	0.0030 N	,,	27	min.(27')
0.0008 N	,,	105	,,	0.0040 N	,,	84	,,
0.0016 N	,,	191	,,	0.0080 N	,,	9	hours.

These figures give the following curve (see p. 442).

Which is the best concentration to compare citrates with the substituted products? When the concentration is sufficiently large *all* salts inhibit milk-curdling. The characteristic of citrate-action is the fact that curdling is prevented in concentrations in which other salts give a scarcely perceptible delay. In general therefore the results of the comparative investigation will be the more correct,

the smaller the concentrations used. On the other hand the difficulty of accurately determining very small lengthenings of curdling-time,



forms another limit. The best concentrations proved to be 1/125 N and 1/25 N; citrates in this concentration practically inhibit milk-curdling, while indifferent salts as sodium chloride, sodium formiate among others show none or very little influence.

In order to find the influence of salt on milk-curdling, 1/80 gram-molecule [1]) of the acid was neutralized with titrated natron, with addition of two drups of litmustincture till the colour was the same as distilled water with the same quantity of indicator.

Then the volume was brought to 50 cc. with distilled water. In this way a neutral solution was obtained, containing $^1/_4$ grammolecule of neutral salt per liter. In the same way or by diluting the $^1/_4$ N. solution $^1/_{20}$ N. solution of the neutral salts was obtained.

The curdling-time was determined as described above; only 2 c.c. of the salt was added instead of the 2 cc. of distilled water. After

[1]) Not 1/80 equivalent, but 1/80 molecule; therefore of a tribasical acid with mol. weight $200 \frac{200}{80}$ gr. were dissolved.

all had been mixed and brought to the right temperature, again $^1/_2$ cc. of diluted rennet was added. [1])

I found the following lengthenings of curdling-time:

a. The alcoholgroup made inactive.				
Acetylcitric acid	$^1/_{125}$ N	$3^1/_2'$	$^1/_{25}$ N	$9^1/_2'$
compared with				
Aconitic acid	$^1/_{125}$ N	$2^1/_2'$	$^1/_{25}$ N	$9'$
Tricarballylic acid	$^1/_{125}$ N	$2^3/_4'$	$^1/_{25}$ N	$9^3/_4'$
Isoallylentetracarbonic acid	$^1/_{125}$ N	$3'$	$^1/_{25}$ N	$9^1/_2'$
b. One Carboxylgroup made inactive.				
Symmetric citricacid-monoamide	$^1/_{125}$ N	$1^1/_4'$	$^1/_{25}$ N	$6^1/_2'$
compared with				
Malic acid	$^1/_{125}$ N	$1'$	$^1/_{25}$ N	$6'$
Tartaric acid	$^1/_{125}$ N	$1^1/_4'$	$^1/_{25}$ N	$6^1/_4'$
Trioxyglutaric acid	$^1/_{125}$ N	$1'$	$^1/_{25}$ N	— [2])
c. The alcoholgroup and one carboxylgroup made inactive.				
Methylencitric acid	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$^3/_4'$
compared with				
Succinic acid	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$1'$
Glutaric acid	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$1'$
Pimilinic acid	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$1^1/_4'$
Suberic acid	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$1^1/_4'$
d. two or more carboxylgroups made inactive.				
Citric acid dimethylester	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$1^1/_2'$
Citrodiamide	$^1/_{125}$ N	$^1/_4'$	$^1/_{25}$ N	$1'$
Citric acid trimethylester	$^1/_{125}$ N	$-^1/_4'$	$^1/_{25}$ N	— [3])
Citramide	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$^1/_2'$
Diaethylester of the citric acid-monoamide	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$^1/_2'$
compared with:				
Monoaethylester of tartaric acid	$^1/_{125}$ N	$^1/_4'$	$^1/_{25}$ N	$1^1/_4'$
Isoamylalcohol	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$^3/_4'$
Glycerine	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$0'$
Erythrite	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$0'$
Mannite	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$-^1/_4'$
Glucose	$^1/_{125}$ N	$0'$	$^1/_{25}$ N	$0'$

[1]) I am indebted to Mr. Ross VAN LENNEP for his valuable help in this part of the investigation.

[2]) Not examined because of the small solubility of the calciumsalt.

[3]) Not sufficiently soluble to be examined in this concentration.

When we consider that the unchanged citrate both in $^1/_{128}$ and in $^1/_{25}$ N solution delays the curdling more than 2 hours, it appears from the above table, that the action of citrate is very much weakened as soon as we substitute one of the active groups of the citricacid-molecule, that it totally stops as soon as we make 2 or 3 groups inactive. In the case of tartaric salts we find the same influence of groups; when the alcoholgroups are made inactive (by acetylation) or one of the carboxylgroups (by esterification), the inhibiting action has disappeared (has fallen to the order of magnitude of all kinds of indifferent substances as is shown by the following figures).

When one group which is substituted, is an alcoholgroup, we get a delay of $3^1/_2'$ with an $^1/_{128}$ N and of $9^1/_2'$ with $^1/_{25}$ N. It seems very remarkable that the compared 3-basic acids without alcohol-group give a delay of the same order of magnitude, viz. $2\frac{1}{2}$—$3'$ with $^1/_{128}$ N and $9^1/_4$—$9^3/_4'$ with $^1/_{25}$ N.

When the one group that has been substituted, is a carboxyl group, we get a delay of $1^1/_4'$ with $^1/_{128}$ N and of $6^1/_2'$ with $^1/_{25}$ N. while with the bibasic acids compared, with 2 carboxylgroups and 1 or more alcoholgroups, these figures are 1—$1^1/_4'$ with $^1/_{128}$ N and 6—$6^1/_4'$ with $^1/_{25}$ N. Here also we find a remarkable agreement.

When 2 or more of the active groups of the citrates are taken away, the lengthening of curdling-time diminishes to 0 à $^1/_4'$ with $^1/_{128}$ N and $^1/_2$ à $1^1/_2'$ with $^1/_{25}$ N, figures which can be obtained also with the compared substances but are in the same order of magnitude as with various indifferent salts. It is therefore better to say, I think, that when 1 or more groups are taken away, the characteristic action of citrate has quite disappeared.

We can get a better insight into the relations here described, if we calculate what would be the concentration of citrate, necessary to give the same lengthening of curdling-time as a substituted citrate. For according to the figures on page 441 this lengthening increases much faster than in proportion to the concentration.

We find then that a lengthening of curdling-time

of $9^1/_4$—$9^1/_2'$ corresponds with a citrate-concentration of 0.0023 N.
„ 6—$6^1/_4'$ „ „ „ „ „ „ 0.0021 N.
„ $1'$ „ „ „ „ „ „ 0.0003 N.

We can state therefore, that the characteristic citrate-action is diminished to about 6% of its original value, when one group has been taken away and is diminished to about 1%, when two groups are substituted. We have found, that an analogous influence on the curdling-time belongs to all salts which possess either three carboxyl-

groups and one or more alcoholgroups. It is the combination in one molecule of these two groups, which each delay curdling-time to a certain extent, which increases this power in the case of citrates so strongly (up to 16 times). It is remarkable that the alcoholgroup is as much necessary for the citrate action, as the carboxylgroups.

Summer of 1911. *Delft, Hygienic Laboratory of the Technical University.*

Biochemistry. — "*The laws of surface-adsorption and the potential of molecular attraction.*" By J. R. KATZ. (Communicated by Prof. J. D. v. D. WAALS). (Introduction).

(Communicated in the meeting of June 1912).

Exclusion of secondary complications.

Surface-adsorption or adhesion plays an important part in biological and biochemical processes, but very little is known of its laws. Especially for the solving of some questions about swelling (imbibition) it is desirable to study this phenomenon more closely. Therefore I have made — although the subject really belongs more to physics than to biochemistry — some researches which are only intended as a first introduction to the study of this subject.

The confusion which is still reigning here, comes, I think, for a large part from the fact, that two different things again and again are mixed up: surface-adhesion at substances which have some other action on the adsorbed fluid (formation af a solid solution, swelling, formation of a chemical compound among others) and uncomplicated surface-adsorption. Among the authors who in the course of the 19th century have studied surface-adsorption, not a single one seems to have carried through this distinction as far as might be wished. And even the two latest investigators of this subject, TROUTON [1]) and FREUNDLICH [2]), still treat the adsorption of water-vapour at glasswool and the adsorption at cotton- or woolfibres, as the same phenomenon; although glass does not take up water between its smallest particles, whereas wool and cotton do this to such an extent that the dimensions of the fibres become perceptibly larger (swelling).

Therefore I think it above all necessary in the experimental study of surface-adsorption, to choose a solid which has no action on the fluid studied. I choose water as the fluid to be investigated,

[1]) Proc. Roy Soc. **77** (1906) en **79** (1907).

[2]) Kapillarchemie.