but $f(V)$ or $f(a)$, as instead of all possibilities only the most probable distribution is considered. When, however, ε_2 and ε_3 are $f(a)$, the above reduction is not allowed, since there $\frac{d\varepsilon_1}{da} = \frac{d(\varepsilon_1+\varepsilon_2+\varepsilon_3)}{da}$ has been put.

So it seems to me that two errors have been made in this reduction, which have yet made it possible for us to arrive finally at the correct result. First of all ε_2 and ε_3 have been taken constant over the ensemble, which would have the result that $\overline{A}$ would be the same as if ε_2 and ε_3 did not exist, because $A = -\frac{d\varepsilon_1}{da}$ is independent of ε_2 and ε_3 for a certain phase. It is just in consequence of the change in phase-distribution which they cause, that ε_2 and ε_3 have influence. In the second place $e^{-\frac{\varepsilon_2}{T}}$ and $e^{-\frac{\varepsilon_3}{T}}$ should now have been put as constant factors before the sign $\frac{d}{da}$, in consequence of which we should, in fact, have again obtained the same value as without ε_2 and ε_3. At all events I think I may be justified in calling in question the strictness with which the method has been applied up to now.

Physiology. — "*Contributions to the study of serum-anaphylaxis.*" By Dr. J. G. SLEESWIJK, Foreign Member of the PASTEUR Institute at Brussels. (2nd Communication). (Communicated by Prof. C. H. H. SPRONCK).

(Communicated in the meeting of February 27, 1909).

To experimentally bring about the phenomenon of serum-anaphylaxis (RICHET) — which with other related forms of changed power of reaction of the organism is denoted by the general name of Allergy (v. PIRQUET) — consequently at least two serum-injections are necessary. For the first, the sensitizing injection, spores of serum are sufficient, whilst, however, at the end of the incubation-stage a larger dose is wanted to bring about intoxication (which, however, is different again, according as the injection is given intraperitoneally resp. subcutaneously, or into the circulation resp. into the brain).

It has of course been asked, whether these two functions, the sensitization and the intoxication, have to be attributed to the same material or to two different constituents of the serum. Now without

entering into the detailed theoretical considerations put up with reference to this, I only wish to point out that in more than one way serum can be deprived of its toxicity for sensitized animals, without at the same time losing its sensitizing power. This also holds good especially for the fluids described in my last communication: serum treated with washed guineapig-blood and the filtrate of dialysed serum, which have both lost their toxicity for sensitized guinea pigs, are nevertheless able to render normal animals sensible to a later injection of horse-serum.

The same is the case with the new method, to be described here after, to deprive the horseserum of his toxicity with the aid of bariumsulphate. Therefore this may be laid down as a general rule. A priori there is no doubt something to be said for the opinion that the sensitizing and the intoxicating principle of horse-serum are represented by two different substances, which apparently may be dissociated with the help of divers biological and physico-chemical processes. I myself, however, should for the present prefer to take an other standpoint, and that for the following reason: taking for granted the fact that for the sensitizing action only traces of serum are required, we may safely assume that in the different methods of destroying the toxic serum principle so much of the active matter is destroyed or fixed, that intoxication can no longer be brought about, but that there is a sufficient quantity left to cause sensibility. Because (and here we have a parallel): for not a single immunebody, whose function can be made to disappear from the serum by heat, there exists a limit for which holds good that after a certain time and for a definite temperature the substance should be altogether destroyed. Even though we can no more prove the presence of such a material by a reaction in vitro, yet the curve, of which the ordinate represents the time and the abscis the temperature, theoretically never reaches zero. And such theoretical possibilities ought to be taken into consideration (as moreover experience has shown in heating horse-serum), when — as appears from serum-sensitization — we can cause a biological reaction with such extremely small quantities of a substance. Therefore I do not see why we should in this respect depart from our generally accepted opinions and why we should not identify the sensitizing antigen with the toxical, if only we bear in mind that the organism reacts upon small doses otherwise than upon large doses. But in this communication we do not want to enter into a more detailed description of the theoretical part of the problem and we will now revert to the facts.

The serum rendered atoxic in one of the above mentioned ways

has now, besides its sensitizing power, retained another quality: it has remained vaccinating, i. e. that sensitized guinea-pigs, treated with it, are already within a few hours immune against an otherwise mortally large dose of horse-serum not treated beforehand. Also fresh serum itself, in the usual toxical dose (4—5 cM³) has vaccinating action, but this is a two-edged sword, because in the majority of cases the animals are killed thereby. BESREDKA, [1]) however, was able to prove, that already with a very small dose of serum (e. g. 0,05 cM³), injected into the abdomen, a sensitized animal could be vaccinated without showing symptoms of disease. Now this fact — it seems to me — may be said to be on a par with the vaccinating action that I found of larger doses of serum rendered atoxical, and at the same time it suggests the following explanation: for the dissensitizing (vaccinating) of an animal rendered sensitized a definite, very small quantity is wanted of a substance found in fresh horse-serum. The latter, administered at once and in larger quantity, acts moreover toxically (sudden dissensitization, shock); a small dose of serum, however, (BESREDKA), or — as we have seen — a larger quantity of serum from which the greater part of this substance has been eliminated, contain still enough of it to dissensitize gradually and without toxical by-actions. In passing it may be remarked here that for a probable practical application in the re-injection of therapeutic sera only such a method is practicable, in which is obtained an atoxical and dissensitizing serum, which at the same time has retained a sufficient quantity of antitoxine-units

Further I have asked myself whether it would be possible, in a simple way to get a closer determination of the generally chemical nature of the antigen of anaphylaxis (by this I mean the toxical and vaccinating matter which I consider identical with the sensitizing principle). Does this material belong to the proteins or to the lipoids?

I have tried to answer this question by means of a method which by BORDET and the present writer in a still unfinished investigation about the causes of specificity is applied in the splitting of antigens into a soluble part and another part insoluble in absolute methyl-alcohol free from aceton. Now we take 5 cM³ of horse-serum (consequently a toxical dose for sensitized guinea pigs), it is dried, rubbed into powder and repeatedly extracted with methyl-alcohol. The alcoholic extract is then, by its being taken up again into a small quantity of alcohol — in which the salts do not dissolve — almost

[1]) C. R. Soc. de Biol. 23 Jan. 1909.

entirely freed from it. Both the extract and the residue are now, each in their turn, treated with 5 cM^3 of physiological salt-solution and thus reduced to the original volume. The extract yields an homogeneous fatty emulsion; the residue with the water forms into a thick liquid, jelly-like mass [1]). Now the first is quite indifferent to sensible guinea pigs; not only is it absolutely unpoisonous, but the animals remain also after the injection of it as sensible to an injection of fresh serum as before. Meantime the part of the serum not soluble in alcohol, wholly injected into sensitized animals, does not give, or hardly gives, rise to symptoms of poisoning, but vaccinates against a later, in itself toxical, injection with normal serum. For this vaccination, however, very small doses of the residue, as BESREDKA found them sufficient for intact serum, do not suffice Therefore the active part of the serum has, through the action of the alcohol, lost together with its toxicity also a part of its vaccinating power. At any rate the proteine-nature of the antigen of anaphylaxis seems in my opinion to be well proved by what is said above.

In continuation of the methods explained in my former communication to eliminate the toxical principle of horse-serum for sensitized guinea pigs (fixation upon pig-blood, dialysis), I am now able to add to them a third process. It is based upon recent and very important investigations of GENGOU [2]). The latter proved among others, that, while water possesses no capacity of suspension for bariumsulphate, in consequence of which this powder rapidly subsides, this sedimentation changes into a dissemination in the presence of some stable colloids. This dissemination is based upon a molecular adhesion, a real adsorption of the colloid by the powder. To the colloidal solutions that show this quality, belongs among others the serum. From an oral communication of GENGOU which has not yet been published it further appeared to me that this investigator had succeeded, by contact of fresh serum with $BaSO_4$ in salt-solution, in depriving this serum of its alexine. This led me to try if perhaps also the toxical principle of horse-serum would be absorbed by this powder. It really appeared to be the case. For this purpose is used a suspension of bariumsulphate in physiological salt-solution containing about 70 m.gr. $BaSO_4$ per 1 cM^3. Three parts of this (or the sediment of it) are treated with 1 part of serum. If, however, instead of physiological salt-solution, distilled water is taken as vehicle for bariumsulphate — or the dry powder, when there is no salt-solution — then the serum

[1]) Neither is able to fix alexine in vitro, in the presence of anaphylactic pig serum.

[2]) "Contribution à l'étude de l'adhésion moléculaire et de son intervention dans divers phénomènes biologiques." Arch. internat. de Physiol. 1908, Vol. VII.

remains toxical. The presence of NaCl-solution is therefore necessary, though a very small quantity (some tenths of 1 cM^3 to the $5cM^3$ serum) appears to be sufficient. Some titrations of diphtheria-serum treated thus, taught me already that the quantity of antitoxines decreases thereby only to a very low percentage. (Conversely, barium-sulphate, which as a dry powder leaves the anaphylactic powder intact, is able, in this same form, to fix the antitoxines). So where the serum-principle, toxic to hypersensible individuals, and the anti-toxical power of antidiptheria-serum can be dissociated according to this process, it seems to me that a priori a practical application thereof is not impossible. It is a purely technical problem to work out these data more closely.

About the reactions of immunity, which are enacted in the hyper-sensible organism, I hope to be able to give further information on a following occasion.

Mathematics. — "*Continuous one-one transformations of surfaces in themselves.*" By Mr. L. E. J. BROUWER. (Communicated Prof. D. J. KORTEWEG).

(Communicated in the meeting of February 27, 1909).

We shall treat in this paper an arbitrary surface, which in the sense of analysis situs is equivalent to a sphere, i. o. w. which is a continuous one-one image of a sphere. We shall submit that surface to an entirely arbitrary continuous one-one transformation in itself and we shall investigate whether this is possible without at least one point remaining in its place.

To simplify we shall consider a continuous one-one image of the surface in a Cartesian plane; the infinity of it then of course forms an exception, because there the continuity of the correspondence is disturbed, and because it must answer as a whole to a single point; for that point we choose a point not remaining invariant in the transformation.

In the Cartesian plane we indicate the points of the untransformed figure by letters without a dash, the corresponding point of the transformed figure by equal letters with a dash. In particular we shall indicate infinity by H and K'.

We now construct in the untransformed figure the system of the circles around K as their centre. These closed curves k lie outside each other, expand from K to H and to each of those curves there