COMBINATION OF GELATIN WITH SOME ORGANIC BASES.

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It is known that the alkaloids are very strong poisons. Their poisoning power can be compared only with that of the glucosides, the hormones, the saponins, HCN, and some bacterial toxins. The alkaloids are sufficiently studied physiologically, and in relation to their chemical structure, but what chemical substances enter in combination with them in the body and in the cell is wholly obscure. Traube explains all the influence of alkaloids through changes in superficial tension. Many investigators have adopted the theory of Overton—that alkaloids, which are all soluble in lipoids, influence the cell through this solution. This hypothesis is supported by the fact that the nervous tissue, so greatly affected by the alkaloids, contains at the same time the greatest amount of lipoids. Other investigators, such as Brunton, Rossbach, and lately Frey and Gürber, think that the alkaloids can combine with the proteins. The latter indicate that the alkaloids can precipitate the proteins. But the conditions of this combination are very incompletely known. Woronzoff says that the liver retains the alkaloids only when Ringer-Locke solution, that contains the alkaloid, is alkalized. When it becomes acid, the liver releases the alkaloid previously retained. He thinks that the acid solution extracts proteins which combine with the alkaloids; but then the question arises,—can the proteins be restored to their former place by making the solution alkaline?

In his book, "Proteins and the theory of colloidal behavior," Loeb gives a very simple method for the investigation of the influence of the pH of the solution on the combination of proteins with anions or cations. He used powdered gelatin that acquired different pH values by putting it for 1 hour in acid solutions of different concentration, then filtering off, washing, and putting in an equal quantity of the
solution of the salt under study. He used AgNO₃, K₄Fe(CN)₆, and other cations and anions, e.g. neutral red, acid and basic fuchsin. In all experiments he came invariably to the same result—the anions are combined only on the acid side of the isoelectric point of the gelatin, the cations only on the basic side.

It seemed of some interest to us to investigate by the same method the alkaloids which form a separate group through their common basic character.

Loeb's method, so very satisfactory in one way, in another way cannot be used for the alkaloids. He determined the combination of anions or cations with the gelatin by its coloring. But the alkaloids are colorless. This forced us to determine them by titration of the filtrates and wash-waters from the known solution of alkaloid, which remained for 1 hour in contact with the gelatin in question. We assembled them all, separately for each sample of gelatin, and titrated...
the alkaloid with NaOH and phenolphthalein in presence of any organic solvent, chloroform, or alcohol for atropine, benzene (benzol) for quinine and strychnine, by continued energetic shaking. The added NaOH releases the alkaloid from its salt, the alkaloid dissolves in the organic solvent, and the mixture remains colorless. A new addition of NaOH forces a new quantity of alkaloid to pass in the solvent, and this continues till the last traces of alkaloid pass into the solvent. The following addition of alkali makes the mixture red. This method gives very good results with solutions that contain only alkaloids, but we possessed a mixture of alkaloids with acid or base, that passed into the solution from previous treating of the gelatin. We determined

their quantity by separate controlling experiments and subtracted or added the number of cc. of NaOH used in the control from the number used in the experiment. The difference represents the quantity of alkaloids in the solution. The difference between this quantity and that added represents the alkaloid quantity combined with the gelatin. The accompanying charts indicate very clearly that gelatin combines with the alkaloids very much on the basic side of its isoelectric point. On the acid side the combination is very little or none. The quinine curve gave apparently a very strange result: in all our experiments on the acid side, we found more alkaloid than was added. We think it is because the titration method

![Graph](image-url)
does not determine the alkaloid itself, but the acid in combination with it that the quantity of this acid is increased on the acid side. Quinine can form two sorts of salt—one with one or two parts of acid; on the acid side of the isoelectric point in this case we possess a mixture of two bases, the monochlorate of quinine and the gelatin—and the other a single acid, the H₂SO₄ in combination with the gelatin. The basic character of the gelatin is more feeble than that of the quinine salt.

![Graph](Image)

**Fig. 3.** Curves for strychnine. Determined by the same method as for atropine.

and the latter subtracts a part of the acid from the former. This process is expressed through the lower part (under 0) of the quinine curve.

But the question arises that perhaps this disappearance of alkaloids from the solution is to be explained by their destruction and not by their fixation. This is very unlikely because strychnine and quinine are both very stable substances, but we think that this supposition
Fig. 4. Curves for quinine. Determined by the same method as for atropine. The curve below 0 indicates the number of cc. of acid extracted by quinine from the gelatin.
Fig. 5. Curves for strychnine and quinine (each average of two estimations). Abscissa, pH determined electrometrically, cc. of alkaloid fixed determined by titration.
FIG. 6. Curves for guanidine (each curve is an average of two estimations). Guanidine determined in the filtrates and wash-waters by the Kjeldahl method; the number of N found divided by 3 (3 atoms of N in each molecule of guanidine) and subtracted from the number of cc. added. Correction given for the N of gelatin.

FIG. 7. Curves for adrenalin (each curve is an average of two estimations). Adrenalin determined in the filtrates and wash-waters by the method of Folin. The studied solution used as standard. No correction for N gelatin (it does not give the Folin reaction). Curves for the difference of adrenalin added and adrenalin found.
can be verified very easily. We tasted the gelatin, because quinine and strychnine are both very bitter. The acid gelatin was not bitter at all even if alkalized, and the basic was bitter. The bitterness grew with the basicity. The gelatin used in experiments with atropine was investigated for its power to dilate the pupil; the acid did not act at all and the basic caused a very distinct and prolonged dilatation of the pupil of young dogs.

But besides alkaloids there exist other bases, the so-called animal bases, and many of them are of a very marked and great significance for the organism. We thought that they should give the same results, and verified this supposition by the same experiment. We chose guanidine, the rôle of which in tetany is so much disputed, and adrenalin, the significance of which in the organism is unquestioned. We determined the guanidine in the filtrates by the method of Kjeldahl—a correction being given for the N from the gelatin itself. As shown by the curve, the results are the same as in preceding experiments. The adrenalin was determined by the method of Folin, the adrenalin solution used being employed as standard, with the same results. Correction for the gelatin was not necessary, since the filtrate from the gelatin used did not give the Folin reaction. But adrenalin is very rapidly destroyed in basic solution, and it was necessary to ascertain that its disappearance from the solution was not caused by destruction, but by fixation. We used a physiological test. The gelatin in question was placed on the conjunctiva of the eye—the basic gave a rapid constriction of the vessels, the acid, none.

We think that the same results will be given by histamine, spermine, and other animal bases, and that the experiments here described give us some right to affirm that alkaloids and the so-called animal bases can combine with the proteins (with gelatin we have had an experiment with a mixture of brain proteins and strychnine with the same result) only on the basic side of their isoelectric point. On the acid side the combination is very slight if any. The same must be true in the living cell. The experiments of Bornstein and Rüter and of Labes, explained by the authors in some other manner, reproduced our results so exactly that this cannot be a mere coincidence. In the experiments of Labes, tadpoles swim in the acid solution of atropine or cocaine without any signs of poisoning; in the basic solution they
perished very rapidly. Neuschlosz in his experiments with veratrine found that a gelatin plate, that retained veratrine from previous treating, releases more alkaloid when placed in an acid solution, than in a basic solution. We think the characteristic curve of muscle contraction after veratrine application can be explained in this manner. The muscle tissue under the influence of veratrine gives very characteristic contracture for a certain time, after which its contraction becomes normal. When the muscle is allowed to rest, its contraction returns to its former character but after some further contractions becomes again normal. We think this can be explained by the fact that contraction of muscle is followed by appearance of lactic acid, which lowers the pH of the muscle below the isoelectric points of its proteins (their isoelectric point is very high according to Weber—6.3 for myogen and 5.15 for myosin), and below this value the protein cannot fix the alkaloid. When the muscle rests, the acid disappears, the pH is raised above the isoelectric point, and the muscle fixes the alkaloid, and the latter influences its contraction.

We think that the experiments of Neuschlosz and Riesser with the heart glucosides can be explained in the same manner with the difference only that these substances in contradistinction to the alkaloids are acid and are combined on the acid side of the isoelectric point. The chemical structure of these glucosides is very insufficiently studied, it is better known only for strophanthin, and it contains in its molecule a lactone of an acid. We think it of some interest to study this question more closely. In a recent number of the J. Biol. Chem. a very interesting communication by Chapman, Greenberg, and Schmidt appeared, who studied the fixation of acid dyes by gelatin, edestin, casein, and deaminised gelatin. They came to the same result as Loeb—that these dyes can be fixed only on the acid side of the isoelectric points of these proteins, and that this fixation possesses a stoichiometric character, and, what is new, stands in a very close relation to the quantity of the diamino-N of these proteins. By deaminising the proteins their combining power for acid dyes can be greatly lowered. We think this is a very strong proof against the adsorption theory.

The same method can give interesting results in the investigation of the toxins, and this we expect to undertake.
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