in connection with preparations falling into this as well as into some other groups, is whether or not it is possible that the standardization of preparations of dubious prophylactic and therapeutic activity may give rise to a fictitious and wholly unwarranted confidence in the preparations. An exception must be made to this generalization, and this of course is with reference to antityphoid vaccine, the proven worth of which has made it necessary to adopt a standard test on its agglutinogenic qualities, a criterion which we realize is not satisfactory, although it is the best we have.

TOXINS AND SIMILAR PREPARATIONS

We consider here diphtheria toxin for the Schick test. Standardization is based on the Minimal Lethal Dose, and to meet the common practice of the day provides for dosages of one fiftieth and one fortieth of the guinea pig minimal lethal dose as the human dose.

Toxin-antitoxin mixture: The mixture is tested for toxicity, both as to its ability to cause death to the test animals within the usual period of four days and its ability to produce paralysis and later death.

POLLEN EXTRACTS

Pollen extracts are on a most unsatisfactory basis and we are now working on serological tests, controlled by clinical observation, in the hope that one may be adopted or developed which may serve as an index to the desensitizing value of the preparation.

TUBERCULIN

This is still another of the preparations which have occupied more of our time and thought than is warranted by their importance from a therapeutic or prophylactic point of view. We are unable to report any successful outcome of the work which has been devoted to this preparation.

VIRUSES

The most important of these is smallpox vaccine, and there is now actually in use at the hygienic laboratory a method (not original with us) which, by the inoculation of rabbits with a series of dilutions of commercial vaccine, enables us to form a rough estimate of the potency of the material. This particular preparation is so susceptible to external influences, particularly to the temperature at which it is kept, that a potency test satisfactory to-day gives no indication whatever of the efficiency of the material a few days later, if it has been kept under conditions unfavorable to the preservation of the virus.

ANTIRABIC VIRUS

The several modifications of the classical Pasteur treatment, which itself is still in use, have all been examined sufficiently to lead us to believe that the various preparations will, when properly used, serve to prevent the development of rabies when applied sufficiently early.

From this hasty survey you will see that the immunological art is very extensively applied in the control of biologic products and that there are ample fields of investigation in this line which, when sufficiently worked, will enable us to place all of the preparations which are of value on a sounder basis than we have for many of them at the present time.

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SOME MODERN PROBLEMS IN LEATHER CHEMISTRY[1,2]

WHEN the literature of leather chemistry of twenty-five years ago is compared with the publications of to-day, one is impressed by the extraordinary progress that has been made in a quarter of a century. The striking feature of the newer papers lies not so much in the results of technical and practical use, although such are to be noted, but first and foremost in the entirely different point of view in the choice and treatment of the problems. The pioneers in leather chemistry, among whom I would especially mention W. Eitner in this connection, have collectively produced a lot of valuable experimental data which they worked up solely from the standpoint of direct practical application. They have opened up an exceedingly fruitful field of experimentation, also quite naturally utilizing contemporary

[1] Translated from German by A. W. Thomas.

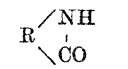
[2] Presented before the Leather Division at the sixty-fourth meeting of the American Chemical Society, Pittsburgh, Pennsylvania, September 4 to 9, 1922.

scientific knowledge. Activity in pure research, however, for the purpose of enlightenment and explanation, entirely unfettered by any thought of practical application, in which the complete structure of modern science is employed, has evidenced itself in the field of leather chemistry only within the past decade. One name joins these two phases of the development of leather chemistry like a bridge—H. R. Procter, who has given us in his "Principles of Leather Manufacture" a large number of very valuable conclusions based on experimental observations, and whose studies on the swelling of gelatin bear the character of the purest scientific research.

How the example set by Procter is being felt by the younger generation and how they are successfully progressing on the road of pure investigation is especially evident in the United States. Such a lively interest in purely scientific studies was unheard of in this field in the past century.

I may be permitted to touch upon a few of the problems which are considered of timely interest by modern scientific leather chemists and to contribute a few remarks to the discussion of these questions which will undoubtedly be brought out in their September, 1922, meeting. My selection in this connection is—our conceptions on the chemical nature of collagen, the mechanism of bating, and what we call the astringency of vegetable tannins.

Concerning the constitution of collagen we know merely what the study of hydrolysis has taught us. From hydrolysis we have a quite exact knowledge of the ultimate split products, the amino acids, but we are only very incompletely informed concerning the nature of their participation in the construction of the collagen molecule. We are indeed justified in assuming that in the main the amino acids are bound together in a peptid-like manner; but this leads us only so far as the peptones, *i. e.,* to those large building stones of which the collagen complex is constructed. We know practically nothing about the manner in which the peptones are bound together. It appears certain that their linkages are fundamentally different from those of the amino acids within the peptone molecule, since pure proteolytic enzymes which cannot effect any decomposition of peptide groups (*e. g.,* pepsin), can break proteins down only to the peptone stage. It is possible that the peptones are combined in a special fashion, which while resistant to peptolytic enzymes (*e. g.,* Erepsin) is vulnerable to the attack of proteolytic enzymes (pepsin). Up to date nothing definite is known concerning the nature of such combination. Plimmer's supposition that it is a question of anhydride-like groupings of amino acids is unproven, and the alternative conception of J. A. Wilson[3] that collagen and gelatin can be represented by the formulas



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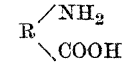

respectively, is not very convincing, since it is difficult to see why the -NH-CO- groups in collagen hydrolyzable by pepsin should not be attacked by trypsin, while in other respects the behavior of the peptid groups is just the reverse.

If the peptone linkage is assumed to be through primary valences, then we are forced to seize upon a kind of linkage entirely different from the peptide groupings, and concerning which, as previously mentioned, nothing is exactly known up to the present. It is possible, however, that the nature of the connections between peptones within the protein molecule is not at all through groupings which depend upon the saturation of primary valences, but that it consists of much looser bonds in which the peptones play an independent rôle. It is conceivable that this loose connection between the peptones becomes more tenuous during swelling of hide perhaps due to the enlargement of the watery envelopes existing around each single peptone molecule. Accordingly it is clear that swelling could be regarded as a preliminary stage in the peptonizing (*i. e.,* decomposition into peptones). It is also plain to be seen according to such a conception that the skin of young animals, rich in water, would be more easily transformed to gelatin than the hide of older animals, and further that the process of liming would become

[3] *J. Am. Leather Chem. Asso.,* (1917), 12, 108.

more difficult to carry out or even be frustrated in purpose upon dehydration (with alcohol) or by drastic desiccation. There are many examples illustrating the point that gel substances are the more readily peptized, the larger the watery envelopes about the single particles and thereby the greater the distance between them. In collagen the peptones may play the rôle of the individual particles, bound together by weaker valence forces than is wont in the case of primary valence. Just what this kind of affinity will be called remains to be seen. In any case, for the purpose of a preliminary working hypothesis, the following assumption may be of service: Peptones are bound to each other and to other smaller complexes in a loose manner, and are somewhat more strongly separated from other similar peptone complexes by larger water shells. During swelling of the protein these loose bonds can become still more tenuous.

The question now arises as to what influence swelling of collagen exerts on the action of enzymes, where the swelling is caused only by neutral salt solutions, and not by acids nor alkalies, in order to exclude the possibility of hydrolyzing action, *i. e.*, splitting of peptide linkages, by H^+ or OH^- ions. Experiments carried out in the Institute of Leather Chemistry at Darmstadt, and which will be reported elsewhere, have shown that the action of trypsin on collagen (white hide powder) depends to a high degree upon preliminary swelling in neutral salt solutions. The following results are especially noteworthy. If collagen is strongly swollen in normal KCNS or KI solutions, trypsin will almost completely dissolve it. It does not matter whether the trypsin has been added with the swelling salt or afterward. This tryptic action is predominately proteolytic, *i. e.*, it converts the protein predominately to peptones or peptone complexes and splits these into amino acids only to a relatively slight degree, which is shown by the fact that the hydrolysis products in the solution give copious precipitates with tannin, while formol titration yields smaller values than in the absence of the swelling salts. It appears then that the decomposition goes only to the peptone or peptone-complex stage and that the peptolytic action of the trypsin is prevented by the salt present. It is further worthy of note that very concentrated solutions of thiocyanates (5 to 8 N) and of iodides (3 N) have an inhibiting effect on the proteolytic as well as on the peptolytic action of the trypsin, and an astringent or shrinking action of the salt solutions runs parallel thereto.

Experiments with chlorates, nitrates, chlorides and sulfates have shown that these salts bring about swelling, decreasing in effect in the order named, and their influence on the action of trypsin is parallel to the decreasing swelling. Experiments were performed where pieces of pelt were treated with trypsin in N/2 solutions of the various salts at 37° C. The trypsin acted most vigorously upon the pelt, in the presence of thiocyanates, and the activities of the other salts followed at considerable intervals in the order iodides, chlorates and nitrates, chlorides and sulfates; the same order that obtained for the swelling power in the absence of trypsin. The effect is scarcely noticeable with chlorides, and in the case of sulfates it is doubtful whether the effect is null or even slightly negative, *i. e.*, whether sulfates exert a weakly inhibitory effect on the proteolytic action of trypsin.

I wish to especially emphasize the fact that the comparative experiments were all undertaken at the same hydrogen ion concentration (electrometrically measured). It is evident that it is not correct to explain the swelling power of an electrolyte solution solely and entirely as a function of the hydrogen ion concentration. The anion of the electrolyte exercises a very pronounced influence, and the Hofmeister series are not yet to be relegated to the class of abandoned fallacies.

What further conclusions may be drawn from the above observations?

First and foremost care must be exercised concerning the presence of salts in the bate liquor. The greater the swelling effect of these salts, then the more dangerous the activity of the trypsin, since it can lead to deep-seated peptization and to heavy damage to the hide.

Further, the various, widely differing observations which have been published concerning the effects of enzymes (especially trypsin) on hide can be understood. Different experimenters have obviously used collagen of very different degree of swelling, and consequently obtained discordant results. It is also under-

stood why collagen from different animal hides and tendons, while not showing any distinct analytical variations, have behaved differently in their ease or difficulty of conversion to gelatin.

It is already on record in physiological textbooks that collagen that has been previously treated with hydrochloric acid will be attacked by trypsin. That this effect is not a function of the hydrolyzing action of the acid, but rather due to the increase in the swelling is evident from the analogous action of neutral salts which bring about swelling of the collagen.

A new question which turns up is worthy of consideration, namely, whether the fixation of tannins is increased by the loosening of the peptones in the collagen-complex, that is, whether an increased development of surface is effected in the hide by the swelling with neutral salts, which would be shown by an increase in the amount of tannins capable of being fixed. Such experiments are contemplated.

I might now turn to another question which stands out prominently in the field of leather chemistry; the character of astringency. We combine with the idea of astringency the conception of tanning intensity, but until recently there has been no means at our disposal to determine comparative differences in degree of tanning action between two tanning agents which showed the same "tannin" content.

Wilson and Kern[4] have attempted to accomplish this through the determination of the tanning velocity, based on the assumption that a certain proportionality exists between astringency and speed of adsorption. Another method could be suggested from the viewpoint that a strongly astringent tannin will be taken up by a surface of weak adsorptive forces, while a tannin of low degree of astringency requires a powerfully adsorbing surface. A fractionation of the tanning material takes place according to the astringency in each liquor, since hide already considerably tanned and therefore of low adsorptive power will take up only the most astringent fractions in the strongest liquors, while fresh untanned hide will take up the mildest, least astringent tannins and even "non-tans." To test this view it is merely necessary to prepare hide powder of varying degrees of tannage, taking care that all soluble matters are washed out of the tanned powders. Treatment of different tannin solutions with these hide powders of varying degrees of tannage should yield information regarding the relationship of the astringent to the mildly acting parts present. Such experiments are now in progress in this laboratory and it will soon be seen whether they lead to useful conclusions. This procedure will by no means touch upon the question of the reason for astringency. Very interesting experiments by A. W. Thomas and S. B. Foster[5] were applied to the elucidation of this problem, and the results render it quite conceivable that astringency is a function of the electrical charge of the colloidal tannin particles. Previous to learning of this research I held the view that astringency was connected with the size of particles of the tannin. In order to test this assumption, it would be necessary to study the distribution of particles according to size in various vegetable tanning materials. Such an investigation has been pursued in this laboratory and will be published elsewhere. A few of the results are briefly as follows:

When a tannin solution is fractionally salted out, the first fraction will contain the largest particles, the later fractions, smaller particles, and in the final filtrate there will be the smallest non-precipitable tannin particles. When the separate precipitated fractions are filtered off, redissolved, and their amounts determined, comparable figures are arrived at which disclose the size relationships of the tannin particles in different tannin solutions. In this way large differences with various tanning materials are manifested. For example, an extract of quebracho wood prepared in the laboratory is rich in large and medium tannin particles and relatively poor in the smallest non-precipitable particles. Sumac is entirely different in that the greater part is composed of the smallest particles and very few large and medium particles appear to be pres-

[4] *J. Ind. Eng. Chem.*, 12, (1920), 465.

[5] *J. Ind. Eng. Chem.*, 14 (1922), 191.

ent. Thus there is a characteristic picture for each tanning material, that certainly is also influenced by the concentration and the manner of preparation of the respective solutions. The effect of age and non-tans on the size of particles is interesting. No two solutions behave alike upon aging. There are those which undergo a very considerable development in size of particles (*e. g.*, quebracho and mangrove) and others, in which this does not happen (*e. g.*, gallotannin, oak wood and chestnut wood). In any case one is not justified in assuming that no gradual change has taken place when a tan solution remains clear (no separation of ellagic acid or phlobaphenes). A solution may remain clear while undergoing an increase in size of particles which will be manifested in its tanning effect.

The effect of non-tans seems in most cases to be an increase in the degree of dispersion or in an inhibition of flocculation, or, in other words, diminution in particle size. This recalls the observation of Wilson and Kern where the speed of tanning was shown to be greatly reduced by gallic acid and suggests a relation between size of particles and speed of adsorption. All non-tans, however, do not behave alike; sumac non-tans, for example, have rather the opposite effect.

A closer examination of this non-tan question has shown that the sugar-like non-tans exert no influence on the size of the tannin particles, but that the phenol-like bodies (gallic acid type) have a pronounced effect. Gallic acid diminishes the precipitable fractions of chestnut tannin and of gallotannin to about one half. Here again exceptions are encountered, for quebracho and sumac are not markedly affected by gallic acid in respect to salting out.

This fractionation of tannin solutions is purely colloidal (according to particle size), and not purely chemical (according to components of different chemical constitution) as proven by the fact that the Lowenthal factor of all fractions is the same. Only in the case of oak bark, which is known to contain a mixture of tannins, one reacting green with ferric salts, the other blue, did fractional salting out effect a separation, although incomplete, of these components. This separation was evidenced by the regular change of the Lowenthal factors and of formaldehyde precipitation. In this treatment the tannin reacting green with ferric ion proved to be of higher dispersity than the blue-reacting tannin.

It yet remained to be tested whether the separate salted-out fractions showed any difference in their other colloidal properties. Viscosity and surface tension were measured with this object in view and compared with the corresponding values of the original tannin solutions. No direct relation between size of particles and viscosity was found, but the surface tension figures did prove to be dependent upon particle size. The first fractions (largest particles) always lowered the surface tension of water to the greatest extent, the decrement decreasing with each further fraction.

The increase in size of particles which ensues if the addition of sodium chloride is maintained under the critical precipitation concentration can likewise be explained as due to lowering of the surface tension. Further, aged tannin solutions also show increased lowering of the surface tension corresponding to increase in size of the particles, with the exception of quebracho. Conversely, there is a distinct correspondence with the fact that all processes or effects tending to decrease the size of particles have the expected effect on the surface tension. Among other things we might mention the diminution of size of particles through short heating of tannin solution, and the especially high degree of dispersion possessed by tannins set free from their lead salts by hydrogen sulfide.

This scanty summary of a few experimental results shows what a fruitful field for colloid-chemical result is offered by our vegetable tanning materials and it is to be expected that many points of practical application will be found when greater light is thrown upon the understanding of the problem of astringency.

The ultimate aim, however, in this and in all other leather chemistry researches is the widening and deepening of our fundamental knowledge of an as yet very undeveloped field.

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