is less expense involved in handling one cutting as compared to several. The four-year stems have developed an ephedrine content which should satisfy commercial demand.

Based on estimates received by submitting samples of ephedra from the four-year lot to various buyers, an acre should produce approximately $303.00 worth of ephedra each four years, or an average of $75.75 per year. It should also be considered that:

1. The plants have been continually disturbed for propagation purposes, and have not yet reached their full production capacity.

2. There is positive evidence that the alkaloid content will increase when the plants are left undisturbed.

3. The intention is to grow ephedra on land which at the present time is unproductive, sub-marginal land.

Future prospects for ephedra cultivation appear quite encouraging.

SUMMARY AND CONCLUSIONS.

Yields of ephedra per acre were determined from stems of various ages. These were assayed and data assembled concerning the commercial aspects of South Dakota grown ephedra. The following conclusions were reached.

1. A suitable assay procedure is recommended.

2. Four-year stems represent the best prospect for commercializing ephedra production.

3. While many phases of the problem remain to be studied, South Dakota can produce Ephedra sinica of commercial value, and especially so in case of a national emergency.

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THE PHARMACOLOGY OF SOAPS.*

BY LEROY D. EDWARDS.[1]

The United States Pharmacopœia, eleventh revision, does not require, as did former editions, that the official soap (Sapo Durus) be made from olive oil. This soap may now be prepared from any oil or mixture of oils so long as it meets several chemical and physical tests as described in the U. S. P. The reasons[1] given for this change were that soaps possessing similar chemical characteristics to a soap made from olive oil may be made from mixtures of various oils, and that there are no chemical means to differentiate between such soaps. This change may be questioned on the ground that soaps made from various oils or mixtures of oils may possess, as determined by our present day methods, chemical and physical properties similar to those of an olive oil soap but may, at the same time, show different physiological reactions, *f. i.*, irritant effect upon the skin.

The following studies of the action of soaps on red blood cells and earthworms are offered as an approach to this problem.
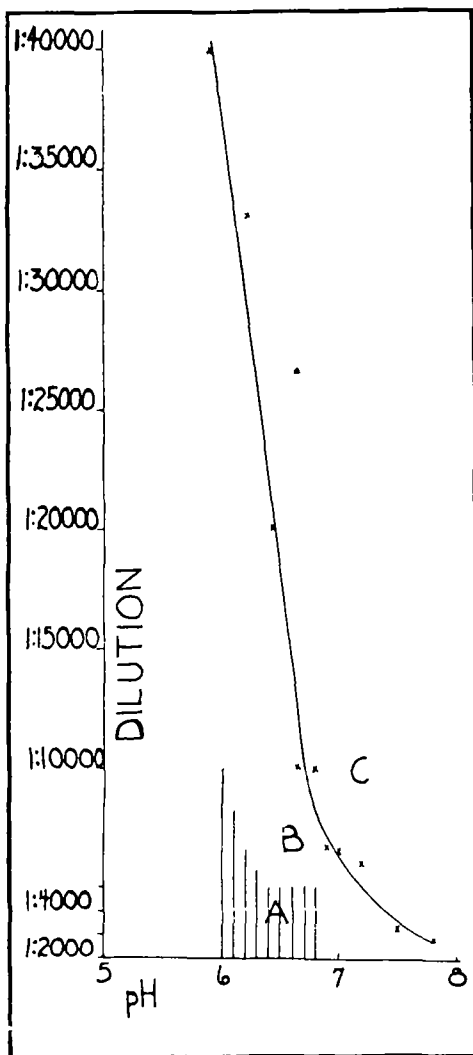
CHEMISTRY OF THE SOAPS EMPLOYED.

The fatty acids—lauric m. p. 42–43 C., myristic m. p. 52–53 C., palmitic m. p. 61–62 C., stearic m. p. 69–70 C.,—were obtained from the Eastman Kodak Company. The oleic and ricinoleic acids were purchased from Kahlbaum. Pure sodium lauryl sulfate was furnished by the Procter and Gamble Company. The absolute purity of some of these acids may perhaps be questioned, but their purity should be sufficient to indicate any possible differences in properties of the various fatty radicles. The sodium and potassium soaps of the solid fatty acids were prepared by adding a computed amount of $N/1$ alkali to a weighed amount of fatty acid suspended in water, and with subsequent warming on the water-bath. After thorough drying in an electric oven the soaps were reduced to a fine powder. The liquid acids were dissolved in absolute alcohol and titrated to neutrality with a strong solution of alkali using phenolphthalein as an indicator. These soaps were also dried and pulverized. In each case the final product was tested for the presence of free alkali and free fatty acid by titrating one Gm. of the soap, dissolved in 50 cc., of boiling alcohol, 95%, which had been previously neutralized to phenolphthalein, with $N/10$ sulfuric acid or $N/10$ sodium hydroxide.

METHOD USED IN HEMOLYTIC TESTS.

One cubic centimeter of each concentration of soap in buffered saline (1 part of buffer mixed with 9 parts of 0.9% saline) was added to 1 cc. of a washed, 5%, human red cell suspension in buffered saline of the same p_H as the soap solution. This mixture was placed in a constant temperature bath for one hour. Most experiments were run at 37 C., some at 25 C. and some at 44 C. In making the readings, the end-point chosen was the dilution which showed no presence of red cells, and was for the most part clear. In all experiments two controls were prepared: (1) soap only in buffered saline and (2) blood cells only in buffered saline. The p_H of the buffered soap solution was checked by means of a quinhydrone potentiometer up to and including a p_H of 8.2.

Graph 1.—Lysis of human RBC by sodium laurate. (A) Zone of clumping. (B) Zone of complete lysis. (C) Zone of no lysis. End-point—greatest dilution of soap causing complete lysis of 1 cc. of a 5% human RBC suspension.

EXPERIMENTAL RESULTS.

The method as outlined above was used to determine the lytic values of the following soaps: sodium laurate, sodium myristate, sodium palmitate, sodium stearate, sodium oleate, sodium ricinoleate, sodium lauryl sulfate, potassium laurate, potassium myristate, potassium palmitate and potassium stearate. One of the striking features of the results obtained, especially with sodium and potassium laurate, sodium lauryl sulfate and sodium oleate is the appearance, over a dilution range and at certain

H-ion concentrations, of three zones of hemolysis (Graph 1).   Zone A, soap concentration highest, is characterized by a cloudiness which, toward the end of the one-hour period, becomes more or less agglutinated.   Under the microscope these clumps are seen as colorless irregular-shaped masses.   Hemolysis probably should be regarded as complete.   Zone B, soap concentration less, is devoid of any solid material and lysis is complete with a clear final mixture.   Zone C, soap concentration least, showed a gradation of some to no hemolysis.   The results for the soaps as a group can best be presented in graphic form (Graph 2).
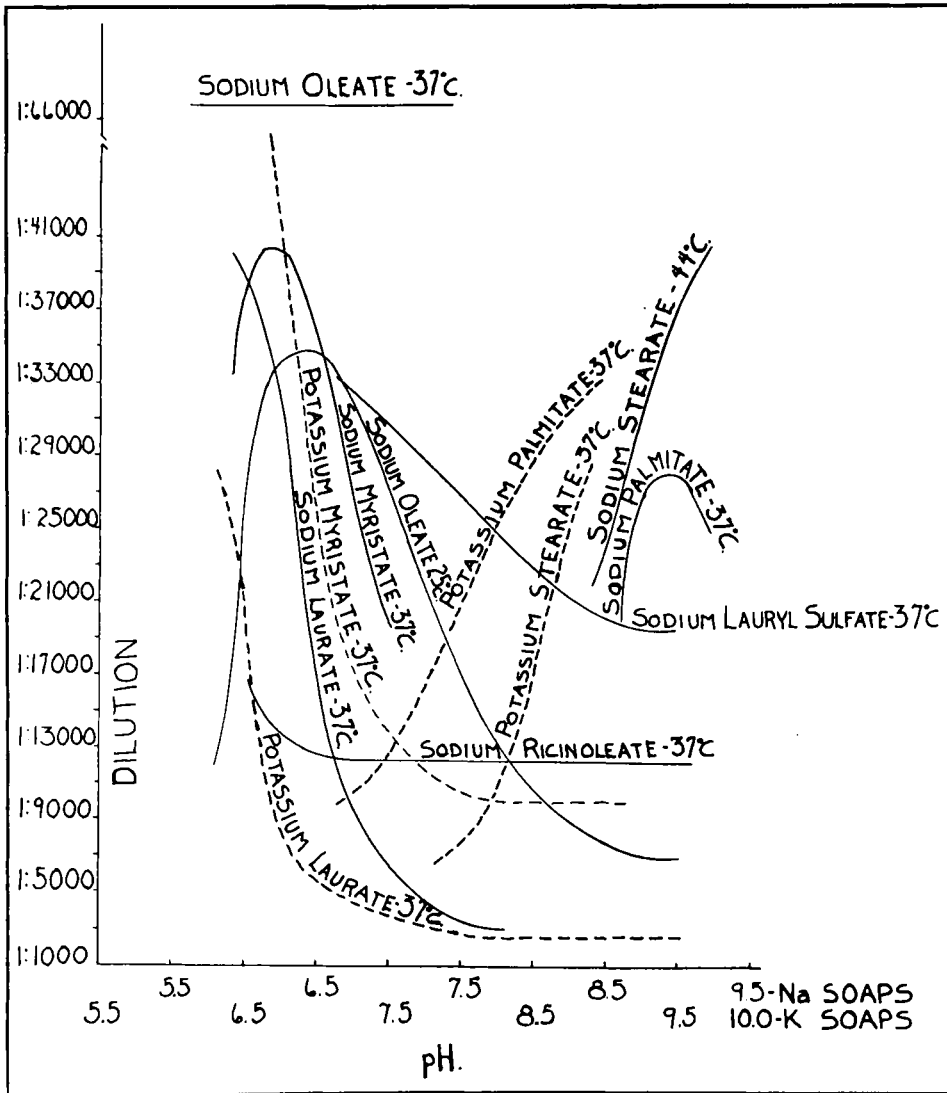
Graph 2.—The hemolytic values of sodium and potassium soaps.   End-point—greatest dilution of soap causing complete lysis of 1 cc. of a 5% human RBC suspension.

DISCUSSION OF RESULTS.

The curve as determined for sodium oleate at 25 C., appears to be the single hemolytic curve that may be considered as a typical curve, and for the purpose of this discussion it will be used as such.   This curve, beginning at the acid side, shows with a decreasing H-ion concentration a rather abrupt rise in hemolytic power which reaches its maximum height at p_H 6.2.   This same

value is found at p_H 6.6, but from there on the curve gradually falls with a decreasing H-ion concentration. The sodium and potassium laurate curves at 37 C., in contrast, show only one leg which falls as the H-ion concentration is decreased—a condition met only with the fall or the right leg of the typical curve. Therefore, it appears justifiable to state that the laurate curves, as determined, are comparable only to the right leg of the sodium oleate curve at 25 C. The curves for sodium myristate and sodium palmitate, although incomplete, exhibit a rise and a fall of hemolytic power in a fashion similar to that described for the typical curve used for discussion. Sodium stearate at 44 C., and potassium stearate at 37 C., present an entirely different situation. Here the curves rise as the H-ion concentration is decreased—a condition noted for the rise or the left leg of the typical curve. On this ground, the statement may be made that only the left leg of the stearate curves has been determined. A relationship between the curves of sodium oleate at 37 C., and sodium ricinoleate at 37 C., both straight lines, and the typical curve is somewhat difficult to demonstrate. However, the sodium ricinoleate curve does tend to rise at the acid side. This suggests that this curve is similar to the extreme end of the right leg of the typical curve. Although there is no direct proof, it does not seem unreasonable to assume that this explanation is also applicable in the case of the straight line curve of sodium oleate at 37 C. This survey of the hemolytic curves as obtained indicates that, over a sufficiently wide range of p_H, each soap should show a hemolytic curve similar in all respects to the typical curve of this discussion. The hemolytic value, beginning on the acid side, would be low. Then with a reduction of the H-ion concentration, the curve, at some certain p_H, would rise to a maximum, and then finally with a further reduction of the H-ion concentration the curve would fall.

If the assumption that the curve for sodium oleate at 37 C., is the extreme end of the right leg of the complete curve is correct, then by comparing this curve with the one obtained at 25 C., it can be stated that the temperature has a marked effect upon the p_H position of the rise in the lytic curve. Further evidence in support of this view is noted in the fact that the rise in the curve for sodium stearate was obtained at 44 C., but could not be determined over the same p_H range at 37 C. These observations indicate that the increase in the temperature is responsible for a shift in the rise of the lytic curves on the p_H scale toward the acid side. In addition the conclusion may be drawn by comparing these two oleate curves that an increase in temperature at a given p_H increases the hemolytic power of the soap. This increased hemolysis may be due solely to an increase in the velocity of the hemolytic reaction, but it is also highly probable that the solubility of the hemolytic component is enhanced.

It is also to be noted that when the different soaps are compared as to their maximum hemolytic power at a given temperature, p_H being disregarded, sodium oleate, a soap of a straight chained unsaturated acid, is the most hemolytic, and that sodium ricinoleate, a soap of a straight chained unsaturated hydroxy acid, appears to be the least hemolytic. The values of the maximum hemolytic powers of the soaps of the saturated fatty acids fall between these two extreme values, and may be arranged in the following order: sodium laurate > sodium myristate > sodium palmitate > sodium stearate. Thus it is apparent that the hemolytic power of a soap bears a relation to the configuration of the fatty acid molecule from which the soap was made. At a given p_H, however, this order, as outlined above, does not hold.

The effect of increasing or decreasing the H-ion concentration at a given temperature produces with the different soaps varying results. Starting at p_H 7, an increase in the H-ion concentration caused sodium laurate at 37 C., to become more hemolytic; sodium myristate at 37 C., gave increased hemolysis up to a maximum value and this was then followed by a slight decrease; sodium oleate and sodium ricinoleate showed no change of any kind; while in the cases of sodium stearate and sodium palmitate the results were considered uncertain. With a decrease of H-ion concentration from p_H 7, sodium ricinoleate curves, again are unchanged; that of sodium palmitate shows a rise with a subsequent slight fall; and finally the curve for sodium stearate at 37 C., was unobtainable.

It is most difficult, with these data, to conclude as to the factor or factors responsible for the observed augmentation and diminution of the hemolytic value of a soap at various p_H ranges. The possible causes worthy of consideration as the active lytic agent of a soap solution are (1) the free alkali of soap solutions, (2) undissociated soap, (3) free fatty acid, (4) fatty acid ion, (5) acid soap. The fact that all aqueous solutions of soaps are alkaline is well known, and many physiological properties of soap solutions have been explained upon the basis of this alkaline reaction.

As far as hemolysis as produced by sodium laurate is concerned, it can readily be demonstrated that the addition of small amounts of alkali causes an actual decrease in the degree of hemolysis. The hemolytic curves for sodium and potassium laurate, sodium and potassium myristate, and sodium oleate at 25 C., all show in the more alkaline ranges a decrease in the hemolytic reaction of these soaps. This evidence surely is sufficient to force the conclusion that free alkali, within reason, in a soap solution is not the predominant lytic component of that solution. A second product of the hydrolysis of a soap is the free fatty acid, and likewise this has been assumed by different writers to be the underlying cause of the hemolytic property of soap solutions. It is, of course, an established fact that the lower fatty acids are, by themselves, markedly hemolytic, and it is only logical, in spite of the slight solubility of the fatty acids found in the common soaps, to suspect the fatty acid content of soap solutions of having something to do with the hemolytic property of those solutions. At the extreme left of the p_H range, the concentration of the free fatty acid will be at its greatest amount, and at the right extreme the amount in comparison will be very much reduced. Then it naturally follows that the amount of undissociated soap present at the left extreme of the p_H range will be very slight, and at the alkaline extreme the undissociated soap will predominate. Applying these conditions to the typical curve, sodium oleate at 25 C., it is noted that at the left side where the fatty acid content is the greatest the hemolytic curve falls sharply. If the fatty acid is responsible for the hemolysis this could not take place. The curve in this range could become a straight line due to the fact that the solution had become saturated with free fatty acid, but it could never fall below the maximum reached if the free fatty acid is the hemolytic component. Sodium lauryl sulfate solutions, where theoretically no fatty acid should exist, has also been shown to be markedly hemolytic. Thus, this evidence makes it very doubtful that the free fatty acid, as such, is greatly concerned in the hemolytic reaction of soap solutions. Turning to the right extreme of the p_H range where the undissociated soap is at its greatest concentration, the hemolytic curve is again found to be greatly reduced. Using the same method of reasoning as employed in the case of the free fatty acid, the undissociated soap likewise can be eliminated as the active lytic agent. This observation of a fall in the lytic curve in the p_H zone where the undissociated soap is at its greatest concentration would also tend to remove the fatty acid ion as the causative agent of hemolysis. If the fatty ion were the lytic component, the soap would continue to dissociate in spite of the reduced H-ion concentration to supply the deficit of fatty ions caused by their removal in the lytic reaction. Thus the curve would rise rather than fall. With this discussion, it becomes apparent that the cause of hemolysis as produced by aqueous soap solutions is some product produced by the hydrolysis of a soap intermediate between the undissociated soap and free fatty acid. This view is forced by the fact that in this intermediate zone on the p_H scale the typical curve reaches its maximum. The acid soaps of aqueous soap solutions appear to satisfy the conditions as developed in this discussion. Although their exact composition is unknown, it is generally agreed that they represent intermediate products between the neutral soap and the free fatty acid. While their solubility is not high, it should be by virtue of the presence of sodium, greater than that of the free fatty acid. This increase of the solubility of the higher fatty acids by their combination with undissociated soap to form a hemolytic acid soap would still leave the primary lytic properties with the fatty acid.

ACTION OF SOAPS ON EARTHWORM SEGMENTS.

Earthworms were decapitated and short segments connected to a lever which recorded the rhythmatic movements of the segment on a smoked drum. After the movements of the segments had been established in air, a bath of 0.5% of soap in water at 25 C. was applied and the movements recorded. The results are given in Graph 3. With sodium laurate the movements were at first increased in rate, but soon disappeared completely. The segment did not respond to electrical stimulation. With sodium stearate the segment continued active throughout, and did respond to electrical stimulation. The actions of sodium myristate, sodium oleate, sodium ricinoleate and sodium palmitate are intermediate between the two above extremes. Controls using air, water and sodium hydroxide solutions of the same p_H as the soap solutions did not greatly interfere with the rhythmic activity of the segment. From these data, the toxicity of the soaps examined for earthworm segments can be stated, starting with the most toxic, as follows: sodium laurate, sodium myristate, sodium oleate, sodium ricinoleate, sodium palmitate and finally sodium

stearate. This classification compares favorably with the results of the hemolytic tests with the exceptions of sodium oleate.

Studies of the actions of soaps on human skin are in progress and will be reported later.
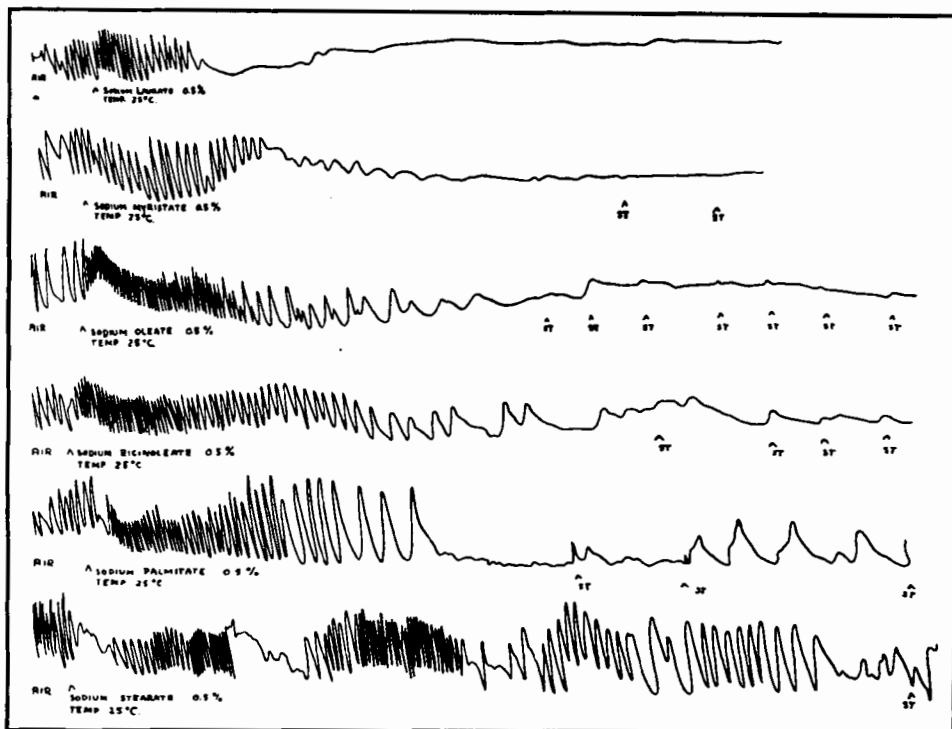
Graph 3.—The action of soaps on earthworm segments.

CONCLUSIONS.

(1)   Evidence has been offered to support the view that the ultimate lytic component of common soap solutions is the fatty acid molecule, and that it is enabled to act as such through the mechanism of some intermediate product of hydrolysis, *f. i.*, acid soap.

(2)   The typical hemolytic curve of a soap shows in the extreme acid range only moderate hemolytic power; then, as the H-ion concentration is reduced, the lytic values rise to a maximum. With a subsequent further reduction of the H-ion concentration, the lytic values are lowered. The rises and succeeding falls in the hemolytic curves of the more soluble sodium and potassium soaps appear at a higher H-ion concentration than do those of the more insoluble soaps.

(3)   When the sodium soaps of the saturated fatty acids are compared as to their greatest lytic values, at a given temperature, they may be arranged in the following order: sodium laurate, sodium myristate, sodium palmitate, sodium stearate. This order does not hold at a given p_H.

(4)   At a given p_H, an increase in temperature enhances the hemolytic reaction of lesser soluble soaps.

(5)   The hemolytic values of potassium and sodium soaps are very similar.

(6) Sodium laurate, of the soaps examined, is the most toxic to earthworm segments. The toxicity of the others are in the order named: sodium myristate, sodium oleate, sodium ricinoleate, sodium palmitate and sodium stearate.

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A STUDY OF THE ASSAYS OF THE POWDERED EXTRACTS OF BELLADONNA AND HYOSCYAMUS.[*][1]

BY HOWARD H. FRICKE[2] AND K. L. KAUFMAN.[3]

In past years, trouble has been encountered in the determination of the alkaloidal content of the powdered extracts of belladonna and hyoscyamus. The potency of these drugs makes it desirable to remedy the difficulties underlying the assays of their extracts. Our work has been carried on in an effort to determine the exact origin of these difficulties, and thus point the way to more satisfactory analytical methods. Most of the experimental studies were made on the pure alkaloids and the powdered extract of belladonna.

The presence of volatile basic substances which were being determined as atropine, was noted by various workers including Goris and Larsonneau (1), Markwell and Walker (2), DeKay and Jordan (3) and the A. D. M. A. Contact Committee's Sub-Committee (4). A few of the volatile bases have been analyzed, but the chemical natures of most of them have not been determined. The A. D. M. A. Contact Committee's Sub-Committee (4) found that the physiological activity of the volatile bases was weak and inconstant.

Various methods of assay have been suggested by different workers in an attempt to eliminate these volatile bases so that the product may be more easily and accurately standardized. At present there is no process which accomplishes this satisfactorily.

Tsakalotos (5) and several other workers found that the mydriatic alkaloids were not decomposed during the assay process as long as they were not exposed to excessively alkaline solutions or were not left in contact with alkaline solutions for long periods. Schousen (6) and several other authors, however, have found that atropine and hyoscyamine are sensitive to water-bath temperature and may be partially hydrolyzed when subjected to water-bath heat. Durrett (7) stated that the hydrolysis of the mydriatic alkaloids could be prevented by the addition of a little dehydrated alcohol near the end of the evaporation of the final chloroform extract. The A. D. M. A. Contact Committee's Sub-Committee (4) has reported that the final heat treatment of the U. S. P. XI assay causes little or no destruction of the alkaloids.

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