

III. SUMMARY.

The solution of fresh posterior lobe according to the method of Burn and Dale has not proved satisfactory in practical use as a standard for the assay of Liquor Hypophysis.

The water-soluble powdered preparation of pituitary glands made by precipitating the concentrated solution of the active principles with acetone is not uniform from preparation to preparation, and therefore requires standardization itself before being available for use as a final standard.

The dehydrated, defatted, powdered preparation made from fresh posterior lobes apparently is uniform from preparation to preparation. It is suitable for use as a standard. Its adoption has, therefore, been recommended.

It also has been recommended that the U. S. P. X require that the finished commercial preparation of Liquor Hypophysis be of such a strength that one cubic centimeter correspond in activity to that yielded by four milligrams of the dried, defatted, powdered gland.

The author wishes to express his appreciation to Dr. H. C. Hamilton, Dr. M. I. Smith, and especially to Dr. Frederic Fenger for the many courtesies extended to him.

BIBLIOGRAPHY.

1. E. A. Schäfer and P. T. Herring, *Phil. Trans.*, 1908, B.
2. H. H. Dale and P. P. Laidlaw, *Jour. Pharm. and Exper. Therap.*, 1912, iv, 75.
3. H. C. Hamilton, *JOUR. A. PH. A.*, 1, 1117, 1912.
4. H. C. Hamilton and L. W. Rowe, *Jour. Lab. and Clin. Med.*, 2, 120, 1916.
5. G. W. Roth, *Jour. Pharm. and Exper. Therap.*, 5, 559, 1913.
6. G. W. Roth, *Bull. Hygien. Lab.*, 1914, No. 100.
7. R. A. Spaeth, *Bull. Hygien. Lab.*, 1918, No. 115.
8. J. H. Burn and H. H. Dale, *Med. Res. Council. Spec. Rept.*, No. 69, 1922.
9. M. I. Smith and W. T. McCloskey, *Public Health Rept.*, March 16, 1923, p. 493.
10. E. E. Swanson, *Jour. Lab. and Clin. Med.*, 9, 334, 1924.
11. E. E. Nelson, *Journ. Lab. and Clin. Med.*, 8, 318, 1923.

DEPARTMENT OF MATERIA MEDICA AND THERAPEUTICS,
UNIVERSITY OF MICHIGAN MEDICAL SCHOOL.

PRELIMINARY REPORT ON THE EFFECT OF FATTY ACIDS ON LINIMENTS AND EMULSIONS.*^{1,2}

BY E. V. KYSER AND FRANK C. VILBRANDT.

In presenting this subject we have made no attempt to determine the critical points of emulsification. The two liniments which we have selected, lime and ammonia, are emulsions, or attempts at emulsions, depending upon the percentage of fatty acids in the oils from which they are made. For convenience we shall refer to them as emulsions. It is our purpose to point out, as they occur to us, certain phases of the effect of fatty acids on emulsions. The Pharmacopœia has no standard for the percentage of free fatty acids which U. S. P. fixed oils may contain and many operators have difficulty in preparing good emulsions by using U. S. P. methods. We believe that some standard for the percentage of free fatty acids of oils

* Scientific Section, A. Ph. A., Asheville meeting, 1923.

¹ Departments of Pharmacy and Chemistry, University of North Carolina.

² Samples of emulsions were made by J. E. Johnson, Ph.C., student at the University of North Carolina.

should be defined and that a more convenient and easy method for making uniform and stable emulsions should be adopted.

The question frequently asked in connection with these emulsion liniments is "why does an old oil produce the best liniment?" The answer usually given to this question is: "an old oil contains free fatty acids which unite with the alkali to form a soap thereby producing an emulsion." This answer can be accepted within certain limits, but applied generally it is erroneous.

The purpose for which a preparation is to be used should, in a measure, determine its composition. In the ninth revision of the Pharmacopœia we have lime and ammonia liniments which are emulsions, if the percentage of free acids in the respective oils used is present in sufficient amounts to form the required quantity of soap to emulsify its oils. These two liniments are used for purposes which are entirely different, and the percentage of free acid in the oils from which they are made seriously affects the physiological value of one and the physical consistency of the other.

Just why the formula for lime liniment was introduced, by the iron workers of Carron, for the treatment of burns is not known, but we may be safe in assuming that this formula of linseed oil and lime water was adopted for three reasons: first, linseed oil of good quality and low in free acid was easily obtained in the vicinity; second, the lime water contains sufficient $\text{Ca}(\text{OH})_2$ to neutralize the small amount of free acid, forms an emulsion and it affords a slightly alkaline medium; third, linseed oil is a drying oil which when oxidized forms a film. This film excludes air and acts as a protective coating. We know that sodium bicarbonate, which is slightly alkaline, is beneficial in the treatment of burns, and that a paraffin dressing which excludes air was found, during the recent war, to be very efficacious for this purpose.

If we accept these reasons for the use of linseed oil and lime water as a treatment for burns, then the use of an old oil would seriously affect the physiological value of the liniment. The free acid present in a greater quantity than that which is neutralized by the $\text{Ca}(\text{OH})_2$ has no physical effect upon the emulsion. This is shown by the table of viscosities and rate of separation of emulsions made from mineral oil and Wesson oil to which we have added various amounts of oleic acid. However, this excess of free acid does afford an acid medium which is not to be desired because it is irritating.

In the case of unsaturated oils the presence of a high percentage of free acid usually denotes rancidity. This is not always true; an oil may be high in acid and not be rancid. Rancidity is usually defined as a state of decomposition in which oxidation has taken place. If oxidation (rancidity) has taken place, then we may expect that the drying value of the oil has been diminished and the property of forming a protective film accordingly decreased. Then by using an old oil we have again defeated the purpose for which the liniment was intended.

There is a vast difference between the physical consistency of an ammonia liniment made from a neutral oil and one made from an oil which is high in free acid. If a neutral oil is used, no emulsion is formed; as the percentage of acid is increased, better emulsions ensue until we reach seven per cent., when we have a semi-solid mass which is too viscous to pour. We have prepared a number of ammonia liniments from Wesson, cottonseed, sesame and linseed oils purchased on

the open market; also, liniments from Wesson and mineral oils to which had been added from one to seven per cent. of oleic acid. The oils bought on the open market containing between two and five per cent. of free acid made good emulsions. The neutral Wesson oil formula gave a cloudy mixture which separates completely in one hour. Wesson oil and mineral oil, to which have been added from two to five per cent. of oleic acid, gave good emulsions; above five per cent. the emulsion became semi-solid. The eighth revision of the Pharmacopœia directs that ammonia liniment be made from cottonseed oil and oleic acid. The percentage of oleic acid was three per cent. of the total emulsion. This formula will produce a good emulsion of proper consistency provided the original oil from which it is made is neutral. However, if the oil should contain four per cent. of free acid, then the addition of three per cent. of oleic acid will produce a semi-solid mass. You can well imagine the embarrassment of the pharmacist who dispenses Ammonia Liniment U. S. P. made from a neutral oil and then refills the order with a liniment made from an oil which contains seven per cent. of free acid. It may be that this occurrence of irregularity in the consistency of the liniments caused the deletion of this formula in the ninth revision of the Pharmacopœia and the adoption of the present sesame oil formula. However, the percentage of free acid in sesame oil varies and we will continue to have this inequality arise from time to time until the Pharmacopœia adopts a standard for an edible oil and then directs the required amount of oleic acid to be used.

Edible oils are comparatively cheap, and if kept in glass-stoppered bottles and protected from light and air, they will remain unchanged for a long period of time. If we adopt an edible oil standard, then any oil which is neutral can be used and the resulting liniments will be uniform and comparatively permanent.

In an endeavor to determine if good emulsions of fixed oils, for internal use, could be prepared—in which such substances as acacia, tragacanth, Irish moss and like emulsifying agents are eliminated—we made a number of experiments, using sodium hydroxide, sodium silicate, various oils bought on the open market, and also Wesson and mineral oils to which we added various amounts of oleic and lactic acids. Our results show that good emulsions can be made by using sodium hydroxide and sodium silicate in the presence of free fatty acids of high molecular weight. Other bases, such as sodium and potassium carbonates and potassium hydroxide, will produce results which are similar. Lactic acid and other fatty acids of low molecular weight will not form an emulsion in the presence of alkali and oil. The sodium lactate formed separates in tiny crystals which tend to break the emulsion.

In view of the fact that various operators have experienced difficulty in making emulsions in which acacia and like emulsifying agents are used and that the oils from which these emulsions are made contain free acids in various amounts—we believe that a solution of these difficulties lies in the use of neutral oils to which has been added the required amount of oleic acid and some alkali which will produce a water-soluble soap as the emulsifying agent. We recommend oleic acid because it can be obtained in a pure state on the open market and because its sodium and potassium salts are good hydrating agents. Emulsions made by using these ingredients are easy to produce and usually permanent. The soap and emulsions are formed simultaneously and if separation should occur reemulsification can be effected by a slight agitation.

It is a well-known fact that water-soluble soaps are very efficient agents for emulsifying fixed oils, essential oils and other substances which are immiscible with water. That such emulsifying agents have not been widely used is probably due to the erroneous popular conception of soaps. They are conceived to be detergents made from materials of questionable source, which are decidedly unfit for internal use. If we consider that many of the oils used in making emulsions for internal use contain free acids, then it is a matter of choice between administering neutralized fatty acids (soaps) or free acids as such. There is no question about the irritating nature of free fatty acids and their chemical activity is proved by the fact that they will combine with alkaline salts, alkaline earths and various metallic compounds. Emulsions in which such substances as acacia is employed are, aside from containing free acid and being difficult to manipulate, subject to fermentation. This fermentation presents a condition which has a tendency to break the emulsion, split the oil, liberate more free acid and cause subsequent decomposition which usually occurs when fermentation takes place.

This popular aversion to the use of soaps for internal use can probably be overcome by referring to the soaps by their chemical names, such as potassium and sodium oleates or the metallic salts of the higher acids of the paraffin series. No objection has been made to the use of sodium oleate as an internal remedy for the treatment of gall stones. If we study the process of digestion, we find that the emulsification of fats in the intestines is caused by the soap formed from the action of sodium or potassium compounds on the free fatty acids. This splitting of fats, by hydrolysis in the presence of the enzymes of the pancreatic juice, is probably carried just far enough to liberate sufficient fatty acids to form the required amount of soap necessary to emulsify the residual fat. Some authorities state that the neutral fat is completely split into fatty acids and glycerol. This is doubtful in view of the time required to completely split fats by the use of live steam and such effective catalytic agents as sulfonated oils. If the total fat consumed were completely split into fatty acids then the fatty acids would be assimilated as sodium or potassium salts and in order to convert these fatty acids into neutral fats, as we find them in the tissue, it would be necessary to break up these soaps and the liberated fatty acids would have to recombine with a glycerol radical. This seems to us a very unusual chemical procedure. Dr. Jean Efront states that the pancreatic juice acts upon fats as a saponifying and an emulsifying agent. The emulsion is produced by the pancreatic juice, owing to the alkaline reaction and to the viscosity of the liquid and not by the action of the enzyme contained therein. The pancreatic juice as well as the products of maceration of the pancreas contain relatively little of the enzyme and the saponification of the substance is always incomplete.¹ Martin H. Fisher, in conjunction with Miss M. O. Hooker, in the discussion of the physical chemistry of emulsions points out that fatty degeneration or fatty filtration is a condition where the fat has become visible or a physical change in which the emulsion has been broken. He draws an analogy between the breaking by acid of a seven per cent. solution of a potassium soap and cottonseed oil with the breaking of a 25% emulsion of fat and liquid which he calls the brain.² Whether soap is present in milk as the emulsifying agent or aids in emulsification will also be considered later in connection with the critical points of emulsification by the use of soaps as the medium.

If the theory of Fisher that fats in tissue are held in a state of emulsion is true,

then we have reason to believe that the water-soluble soap formed in the intestines is the emulsifying agent or aids in the emulsification of fats that occur both in the tissue and in milk.

VISCOSITIES OF EMULSIONS.

Modified Sprengel Viscometer 20° C.

	No.	Composition.	Viscosity (in seconds).	Separation of components.
A	1.	Lime Liniment + Mineral Oil + 1% Oleic Acid	39.0	1 hour
	2.	Lime Liniment + Mineral Oil + 2% Oleic Acid	38.5	1 hour
	3.	Lime Liniment + Mineral Oil + 5% Oleic Acid	38.0	1 hour
	4.	Lime Liniment + Mineral Oil + 7% Oleic Acid	39.5	1 hour
	5.	Lime Liniment + Mineral Oil + 10% Oleic Acid	38.5	1 hour
B	6.	Lime Liniment + Wesson Oil	60.5	2 minutes
	7.	Lime Liniment + Sesame Oil	223.0	+++
	8.	Lime Liniment + Linseed Oil	227.0	+++
	9.	Lime Liniment + Cottonseed Oil	301.0	48 hours
C	10.	Ammonia Liniment + Mineral Oil + 1% Oleic Acid	23.5	+++
	11.	Ammonia Liniment + Mineral Oil + 2% Oleic Acid	24.5	48 hours
	12.	Ammonia Liniment + Mineral Oil + 5% Oleic Acid	63.0	+++
	13.	Ammonia Liniment + Mineral Oil + 7% Oleic Acid	Too heavy	+++
	14.	Ammonia Liniment + Mineral Oil + 10% Oleic Acid	Too heavy	+++
D	15.	Ammonia Liniment + Wesson Oil + 1% Oleic Acid	30.5	5 hours
	16.	Ammonia Liniment + Wesson Oil + 2% Oleic Acid	57.5	+++
	17.	Ammonia Liniment + Wesson Oil + 5% Oleic Acid	275.0	+++
	18.	Ammonia Liniment + Wesson Oil + 7% Oleic Acid	Too heavy	+++
E	19.	Lime Liniment + Wesson Oil + 1% Oleic Acid	30.0	+++
	20.	Lime Liniment + Wesson Oil + 7% Oleic Acid	33.0	48 hours
F	21.	Ammonia Liniment + Wesson Oil	60.0	1 hour
	22.	Ammonia Liniment + Sesame Oil	40.0	+++
	23.	Ammonia Liniment + Linseed Oil	45.5	+++
	24.	Ammonia Liniment + Cottonseed Oil	30.5	+++
G	25.	Mineral Oil + 1% Oleic Acid + 1% Na ₂ SiO ₃	47.0	+++
	26.	Mineral Oil + 2% Oleic Acid + 2% Na ₂ SiO ₃	30.0	24 hours
	27.	Mineral Oil + 5% Oleic Acid + 5% Na ₂ SiO ₃	252.0	24 hours
H	28.	Cottonseed Oil + 3% Na ₂ SiO ₃	24.5	5 hours
	29.	Cottonseed Oil + 5% Na ₂ SiO ₃	60.5	6 hours
J	30.	Wesson Oil + 1% Oleic Acid + 1% Na ₂ SiO ₃	30.5	15 hours
	31.	Wesson Oil + 2% Oleic Acid + 2% Na ₂ SiO ₃	—	24 hours
K	32.	Wesson Oil + 2% Oleic Acid + 2% Na ₂ SiO ₃	30.0	48 hours
	33.	Wesson Oil + 5% Oleic Acid + 5% Na ₂ SiO ₃	—	48 hours
L	34.	45% Wesson Oil + 5% Oleic Acid + 1.2% Na ₂ SiO ₃	27.5	48 hours
	35.	45% Wesson Oil + 5% Oleic Acid + 0.6% NaOH	18.0	2 hours
	36.	45% Wesson Oil + 5% Lactic Acid + 1.2% NaOH	Seps. before run	3 seconds
	37.	Emulsion of Sesame Oil + Acacia	59.5	+++
	38.	Emulsion of Cottonseed Oil + Acacia	117	+++
	39.	Emulsion of Wesson Oil + Acacia	133	+++
	40.	Emulsion of Linseed Oil + Acacia	91	6 hours
	41.	Emulsion of Mineral Oil + Acacia	230	+++

+++ Preparations thus marked showed no separation at end of 72 hours.

It seems evident from the tables of viscosity and rate of separation that there is a limit to the amount of sodium silicate which can be added as an emulsifying

agent. We had difficulty in arriving at true viscosities because of the separation of some of the emulsions during the time of running and the interference of external adhesion. We reduced this effect to a minimum, as far as we were able, by the use of a specially constructed viscometer using a water-jacketed 100-ml. pipette.

Discussion of Result.—It is evident from a survey of Series A(1-5) that an increase of oleic acid content beyond that which is neutralized by the lime in which there is no increase in the amounts of calcium oleate formed, does not influence the internal cohesion and external adhesion of the mixture. The outside influence of rapidity in separating into watery and oily components easily overshadows any influence increasing amounts of calcium oleate may exert. This statement is verified in Series C (10-14) wherein a more perfect emulsion results with ammonia than with lime and an increasing amount of ammonium oleate increases the viscosity. The internal cohesion of the particles of the emulsion overbalances the external adhesion. The emulsion leaves glass surface fairly clean. To begin with, an ammonium oleate mixture flows very freely in the presence of a mineral oil but slight increases in the oleate rapidly increase the internal cohesion of the particles.

Substituting Wesson oil for the mineral oil a much higher viscosity results when compared with those mixtures containing the same percentage of oleates. (Compare Series D and C.) This increase is due to an increase in the external adhesion of the mixture, due to the presence of Wesson oil. In the case of the lime oleates with Wesson oil (see Series E) a reduction in viscosity results, due to a counter-acting influence of the external adhesiveness of the particles and their internal cohesion, yielding a more or less watery or free-flowing product.

Comparing Series B and F, liniments of lime and ammonia, using various oils, Wesson, cottonseed, linseed and sesame, we obtain an insight into the difference in physical properties of the ammonium and calcium oleates. Calcium oleates show a lessening effect on the viscosity, but the presence of much free vegetable oil greatly increases the viscosity due to external adhesion.

That mineral alkalis other than ammonium and calcium can be used is shown by the fact that additions of small amounts of sodium silicate produce some very fine emulsions (Series C to K, inclusive). There is a maximum amount of oleic acid that can be added to sodium silicate and mineral oil that will increase the viscosity of the mass.

Sodium silicate itself having a high external adhesiveness will increase the viscosity with increasing amounts of the silicate. On the whole the results with sodium silicate do not compare as favorably as those with ammonia, since the stability of the emulsions in the latter case is much greater. (Compare G, H, J, with C, D.)

Straight oil emulsions with acacia have a good stability, the internal cohesion and the external adhesion being much higher than the general run of the other series.

Lactic acid cannot be substituted for oleic acid, since it does not give a soap, the calcium lactate formed separating in tiny crystals and tending to break up the emulsion. The presence of a small amount of soap protects the colloids and gives a more stable emulsion.

BIBLIOGRAPHY.

1. Dr. Jean Efront, "Enzymes and Their Applications," John Wiley and Sons. 1904.
2. Martin H. Fisher and M. O. Hooker, "On the Physical Chemistry of Emulsions and Its Bearing on Physiological and Pathological Problems," *Science*, March 31, 1916.