

Collect the filtrates and evaporate in a tared evaporating dish to 3-4 Gm. While warm transfer the residue to a strong test-tube, cool and add 5 Gm. ether. Stopper with a cork. Shake thoroughly and set aside. No needle-shaped crystals should form in the ether or on the walls of the test-tube, even after standing several days.

U. S. PHARMACOPŒIA X.

Boil 10 Gm. of powdered Rhubarb for fifteen minutes with 50 cc. of diluted alcohol under a reflux condenser, filter and concentrate to 10 cc. Cool, shake with 15 cc. of ether, and set aside for twenty-four hours: Yellowish, prismatic crystals should not form (Rhapontic Rhubarb).

ANALYTICAL DEPARTMENT OF THE BROOKLYN LABORATORIES,  
E. R. SQUIBB & SONS.

BIOASSAY OF CAPSICUMS AND CHILLIES I.\*

BY JAMES C. MUNCH.<sup>1</sup>

Capsicum is defined in U. S. Pharmacopœia X (31) as "the dried ripe fruit of *Capsicum frutescens* Linné (*Fam. Solanaceæ*), grown in Africa." In addition to the commercial varieties imported from Africa, under the trade names Zanzibar, Mombassa and Sierra Leone, a number of other species have been developed in Japan and India, the United States and Mexico. Capsicum is used internally as a condiment and carminative and externally as a counterirritant (23).

A number of products have been isolated from capsicum and believed to be the active principles. Heydenreich (7) reported, in 1858, that two oily, non-crystallizable substances differing in alcohol solubility were the active constituents. The sharp tasting principle was designated "capsicin" by Bucholz (2) and by Landerer (8), but this name has been applied to the ethereal extract (3), as well as to the alcoholic extract (30). Buchheim (1) claimed, in 1873, that a dark red oil was the pungent principle. Three years later Thresh (28) stated that the active principle was "capsaicin," which is apparently identical with a product "capsicutin" isolated by Morbitz (12). Micko (11), Meyer (10) and Strohmmer (27) established that the crystalline constituent, "capsaicin," was the active pungent and irritant principle. Micko announced the formula to be  $C_{18}H_{28}NO_3$ , but Nelson in a series of papers (13, 14, 15, 16) reports his experiences in isolating crystalline capsaicin and determining its structure. He obtained 50 Gm. of recrystallized capsaicin from 50 pounds of cayenne pepper. Study with this material showed that it was methyl-nonenyl-vanillyl-amide, with the formula  $C_{18}H_{27}NO_3$ . He synthesized a number of pungent substances of similar composition.

According to various authors (3, 29) the capsaicin content of various species of capsicum ranges from 0.01 per cent to 0.07 per cent or higher. Micko reports that *C. fastigiatum* contains twenty times as much capsaicin as *C. annum* (29). Morbitz (12) stated that *C. fastigiatum* contains 0.05 to 0.07 per cent of capsaicin.

\* Scientific Section, A. P. H. A., Rapid City meeting, 1929.

<sup>1</sup> Grateful acknowledgment is made to H. H. Crosbie for pharmacognostic identification of the products used in this investigation; to C. Jelleff Carr for the preparation of some of the solutions tested; and, finally, to the 100 collaborators who tested weird drinks in connection with this investigation.

From the details given in their reports it appears that these figures represent the yield of material obtained by the method employed and may be much less than the true capsaicin content.

No chemical method for the determination of capsaicin in capsicum has been quantitatively satisfactory. Chemical methods for the detection of capsicum in food or drug products have not given satisfactory results. Methods for the detection of capsicum in ginger ale were developed by LaWall (9) and by Nelson (13), which were essentially identical in principle. The ether extract was saponified with alcoholic KOH, the residual solution acidified and extracted with ether. Evaporation of the ether left capsaicin which gave a sharp taste on the tip of the tongue. Stary (24) showed that the sharp taste of paprika, pepper and ginger is due to stimulation of the endings of the heat nerves in the tongue. Heubner (6) tested irritants upon the corneal nerves and by their power to cause local reaction and vesication on the skin. He found that the pepper products were purely nerve poisons and did not cause dilatation of the blood vessels.

When chemical methods were found to be inadequate attention was directed toward the possibility of developing physiological methods for standardization. The irritant effect upon the skin did not lend itself readily to quantitative results, so attention was directed to the possibility of organoleptic assay. A quantitative test and standard were first published, so far as I can learn, by Scoville (21) in 1912, although he has informed me that he was not the first to test capsicum by taste (22). His original method was to macerate one grain of ground capsicum over night with 100 cc. of alcohol, filter and dilute with sweetened water to the proportion just giving a distinct weak pungency on the tongue. No relation was found between the color or the fat content of oleoresins and the pungency. Tests of commercial materials gave the desired pungency reactions at the following dilutions: Japan chillies, 1:20,000 to 1:30,000 (50 to 30 mg. per L.); Zanzibar capsicum, 1:40,000 to 1:45,000 (25 to 22 mg. per L.); Mombassa, 1:50,000 to 1:100,000 (20 to 10 mg. per L.). Many oleoresins showed pungency at 1:100,000 (10 mg. per L.) although there were some which gave a definite reaction at 1:150,000 (6.7 mg. per L.). He stated that the physiological assay was the only satisfactory method for oleoresin of capsicum.

Gathercoal and Terry (5) made a study of capsicum in connection with the monograph for U. S. Pharmacopœia X, and suggested certain modifications in technic. One cc. of the alcoholic solution from extracting 1 Gm. of ground capsicum with 50 cc. of alcohol is diluted to 250 cc. with distilled water. One cc. of this dilution added to four cc. of 12% sucrose should be as pungent as 5 cc. of 1:10,000,000 capsaicin. Capsaicin at 1:10,000,000 (0.1 mg. per L.), African capsicum at 1:75,000 (13.3 mg. per L.) and piperine at 1:100,000 (10 mg. per L.) are reported to be equally pungent. Their detailed results are given in Table I.

Wirth and Gathercoal (32) concluded that dilution to isopungency gives reliable results. A sample of capsaicin obtained from the Bureau of Chemistry, U. S. Department of Agriculture (Nelson's capsaicin) gave a threshold at 1:10,000,000 (0.1 mg. per L.). Two of a series of five commercial chillies showed thresholds at 1:45,000, one at 1:55,000 and two at 1:70,000 (corresponding to 22, 18 and 14.3 mg. per L., respectively), compared with the U. S. Pharmacopœia standard of 1:70,000 (14.3 mg. per L.).

TABLE I.

Sample no.	Material.	Dilution.	Pungency.	Concentration, mg. per L.
1	Paprika	$\left\{ \begin{array}{l} 1:25,000 \\ 1:50,000 \end{array} \right.$	Distinct	40
			Very slight	20
8	Capsicum, East Indies	1:50,000	Plainly perceptible	20
3	Capsicum, East Indies	1:75,000	Very decided	13
15	Capsicum, Japanese	1:50,000	Plainly perceptible	20
16	Capsicum, Japanese	1:50,000	Plainly perceptible	20
11	Capsicum, West African	$\left\{ \begin{array}{l} 1:50,000 \\ 1:75,000 \end{array} \right.$	Hot	20
			Decided	13
12	Capsicum, Zanzibar	1:75,000	Decided	13
13	Capsicum, Mombassa	1:75,000	Decided	13
	Piperine	$\left\{ \begin{array}{l} 1:50,000 \\ 1:100,000 \end{array} \right.$	Hot	20
			Decided	10
	Capsaicin	$\left\{ \begin{array}{l} 1:1,000,000 \\ 1:10,000,000 \end{array} \right.$	Very pungent, burning	1
			Decided	0.1

In a personal communication (22) Dr. Scoville informs me that individuals will differ greatly in susceptibility and that the daily variations of the same individual are often marked. He required a positive reaction from four out of five men before passing any product. He also points out that the liquid should be allowed to trickle down the throat or swallowed slowly, and not gulped down, to give most satisfactory results. Some capsicums produced a tingle at once which quickly disappeared, whereas others develop their effects more slowly and the pungency persists. The African chillies are usually more pungent. Samples showing reactions at 1:100,000 (10 mg. per L.) are not uncommon and little trouble is had in securing a 1:70,000 capsicum.

DuMez (3) reports a somewhat different method for the physiological evaluation of oleoresin of capsicum. One drop is heated on a water bath with 5 cc. of normal KOH and then diluted to 100 cc. with water. Five cc. of this aqueous solution is placed in a 1000-cc. glass-stoppered, graduated cylinder and diluted with successive 100-cc. portions of water, the dilution being tasted after each addition. The highest dilution giving a distinctly perceptible pungent taste is determined and compared with results obtained on a standard preparation. All of the samples prepared by DuMez were distinctly pungent in dilutions of 1:250,000 (4 mg. per L.). He suggests a standard of 1:200,000 for the oleoresin (5 mg. per L.), stating that the standard of 1:150,000 (6.7 mg. per L.) was rather low.

Wasicky and Klein (33) attempted to develop a quantitative method by applying one drop of a hydro-alcoholic solution of capsaicin, or an alcoholic extract of crude capsicum to the tip of the tongue. They found that the threshold of capsaicin was 1:1,923,076; for the thirty-five crude drugs examined, the thresholds range from 1:263 to 1:13,510, corresponding to capsaicin content from 0.013 per cent to 0.70 per cent, respectively.

A number of qualitative investigations have been made in an effort to connect some definite chemical structure with pungency. Products have been tasted in pure form, in aqueous or alcoholic solution, or ground with flour. Crystalline piperine has been reported to have only a slightly sharp taste, whereas the alcoholic solution has a very sharp taste (17, 18, 19, 25, 26). Riccomanni (20) has shown that the piperidine nucleus is not necessary as a number of methyl

ketones, including phenyl-methyl ketone, have characteristic sharp or pungent tastes. Of the various materials which are reported to be pungent piperine has been found to resemble the pungency of capsaicin most closely, and appeared most suitable for use as a standard.

#### STUDIES OF U. S. PHARMACOPŒIA METHOD.

The bioassay method outlined in U. S. Pharmacopœia X is as follows:

Mix well 1 Gm. of powdered (*a*) capsicum in 50 cc. of alcohol (*b*) in a stoppered flask and macerate for twenty-four hours. (*c*) Dilute 0.1 cc. of the clear, supernatant liquid with 140 cc. of distilled water containing 10 per cent sucrose (*d, e*): 5 cc. of this dilution swallowed at once will produce a distinct sensation of pungency of capsicum in the throat of at least two out of three individuals (*f*).

Two-tenths gram of oleoresin and 10 cc. of tincture of capsicum are substituted for 1 Gm. of powdered capsicum in the respective tests, the other details being the same.

Specific attention has been directed to various details of this method as indicated by the letters in parenthesis inserted in the foregoing description. As a result of these investigations a modified method has been developed which has been very satisfactory in the study of pungency.

(*a*) The official method does not specify the fineness of the powdered capsicum. Because of difficulties in grinding uniformly I have performed experiments on only two sizes—the coarse powder (No. 20) and the moderately coarse powder (No. 40). No difference in pungency was found between these two sizes. It is recommended that capsicum in coarse powder be specified for this test.

(*b*) The official method specifies extraction with alcohol which means a solvent containing about 95% of ethanol by volume. In a series of tests upon four preparations parallel experiments were conducted using 95% and 48% alcohol and no difference in pungency was found. The use of 95% alcohol gives a clearer solution and it was felt that it might be preferable. Accordingly the use of 95% alcohol is recommended.

(*c*) Judging by the rate at which the coloring matters were extracted from capsicum and the fact that capsaicin is readily soluble in alcohol, it was believed that the time of maceration specified as twenty-four hours in the official method might be shortened. Accordingly, a series of experiments were undertaken in which the period of maceration was three, six, eighteen, twenty-four hours, and two and three days. The same pungency was found in all samples at the end of three hours as at the end of longer periods of extraction. (See Table II.) In some experiments complete extraction was obtained in less than three hours but in others the result after one hour was somewhat lower than at the end of the three-hour period. It is recommended that the time limit in the official method be shortened to three hours.

(*d*) The official method obtains the extractives of one Gm. of powdered capsicum in 50 cc. of alcohol and dilutes 0.1 cc. of this liquid, representing 2 mg. of crude drug, with 140 cc. of 10% sucrose solution. This gives a final dilution representing 14.3 mg. of crude drug per L., a value not suited for direct mathematical comparison. Glass-stoppered apparatus having a capacity of 140 cc. is not readily available. In this investigation all dilutions were made in glass-stoppered 100-cc. volumetric flasks or graduated cylinders. The alcoholic extract was made to represent 20 mg. of crude drug per cc. Since the final dilutions were made up to 100 cc. the results obtained were very easily translated into milligrams per liter by multiplying the volume of alcoholic extract taken by the quantity  $10 \times 20$ , or 200. A simple calculation gives results in other terminology. Expression of values in terms of milligrams per liter appeared preferable as being more readily understood and more suited for comparison of different samples of crude drugs as well as in comparing the pungency of galenicals.

(*e*) The use of 10% sucrose solution as a diluent is specified in the official method. A series of experiments were undertaken in which 5%, 7.5%, 10% and 15% sucrose solutions were

used. It was found that the same degree of pungency was obtained irrespective of the concentration of sucrose employed, but that smaller differences in concentration could be detected in 10% sucrose than in any of the others. It is recommended that the final dilutions be made in 10% sucrose solution.

(f) The official method prescribes that 5 cc. of this dilution will cause a distinct sensation of pungency in the throat of at least two out of three individuals. This is not a very definite standard since by proper selection of individuals widely divergent results may be obtained on a quantitative basis. It may be satisfactory for a qualitative test and will serve to reject material which is greatly under strength. However, the great variation between different individuals and in the same individual at different times makes it advisable to use a definite standard to determine the threshold sensitivity of the humans on test. Attention was directed toward several substances as possible standards. It was found that crude capsicum changes in pungency with age and accordingly it was dismissed. The variation in potency of commercial oleoresins was too large to recommend the indiscriminate use of an oleoresin. Through the kindness of E. K. Nelson a sample of capsaicin was obtained. The results obtained in testing this sample are reported later in this paper. This would serve as a very suitable standard if adequate supplies could be obtained. However, it is very scarce and too difficult to obtain in sufficient quantity to be recommended as a primary standard of reference.

After several other substances had been tested and rejected for one reason or another it was found that commercial piperine met the requirements. A sample of piperine which had precipitated from oleoresin of black pepper was recrystallized once and twice from acetone. Another sample was purchased upon the open market. The melting points of the original samples were 134° to 135° C. After one recrystallization the melting point had fallen to 132° and after a second recrystallization to 129°. (The literature specifies 129° to 130° C.) Alcoholic solutions of all four samples were found to have the same degree of pungency. Fifteen mg. per L. failed to show any pungency, and 16 mg. per L. showed distinct and definite pungency upon ten individuals. Accordingly, it is recommended that piperine be adopted as the standard for determining the threshold of individuals testing the pungency of capsicum and its preparations. It is recommended that 16 mg. per L. be selected as the threshold concentration. In case a stronger concentration is required to produce the minimum degree of pungency selected as a satisfactory end-point, corresponding changes should be made in the thresholds for crude capsicum and for its preparations. This should be done in the same way that changes are made in the assay of the digitalis group when ouabain is employed for standardizing frogs.

Based upon the results obtained in this investigation the following procedure is suggested for the bioassay of capsicum.

Shake 1 Gm. of coarsely powdered capsicum with 50 cc. of alcohol in a stoppered flask for 3 hours. Dilute 0.1 cc. of the clear, supernatant liquid with 100 cc. of 10% sucrose solution. Five cc. of this dilution swallowed during 5 seconds will produce the same degree of pungency in the throat as 5 cc. of 10% sucrose solution containing 16 mg. of piperine per L. In case 16 mg. of piperine per L. does not produce satisfactory pungency, the threshold concentration should be determined and corresponding alterations made in the standard for capsicum (0.1 cc. per 100 cc. or 20 mg. per L.).

#### BIOASSAY OF CAPSICUM.

Using this modified method the threshold concentration of a number of capsicums just causing definite pungency in the throat of the author and of ten collaborators have been determined. Results are given in Table II.

TABLE II.  
PUNGENCY OF CAPSICUM.

PA no.	Product.	EXTRACTION CONCEN. Alcohol.	Time.	Minimum effective conc., Mg. per L.
480	Zanzibar	95%	24 hrs.	20
481	Sierra Leone	95%	24 hrs.	20
695	Sierra Leone	95%	24 hrs.	30
496	Annum	48%	24 hrs.	30
499	Annum	95%	24 hrs.	30
692	Japan Chillies	95%	1 hr.	30
692	Japan Chillies	95%	3 hrs.	30
692	Japan Chillies	95%	6 hrs.	30
692	Japan Chillies	95%	24 hrs.	30
688	Louisiana sports	95%	24 hrs.	200
689	Louisiana longs	95%	24 hrs.	400
687	Tobasco	95%	24 hrs.	100
682	Short Bombay	95%	24 hrs.	100
683	Long Bombay	95%	24 hrs.	140
649	Commercial sample No. 1	95%	3 hrs.	30
			24 hrs.	30
			2 days	30
650	Commercial sample No. 2	95%	3 hrs.	20
			24 hrs.	20
			2 days	20
651	Commercial sample No. 3	95%	3 hrs.	60
			24 hrs.	60
			2 days	60

The data on Japan chillies and on the three commercial samples are reported to show that extraction is complete within three hours if not sooner. The products reported in this table, with the exception of the last three, were obtained in a whole unground condition through regular drug channels. The last three products (PA 649, 650, 651) were submitted by three drug brokers in response to orders for "ground capsicum U. S. P." Sample No. 3 was vivid red in color, the other two orange-brown. Analyses upon a number of other samples not included in this table suggest that capsicum may be obtained giving a definite pungency in alcoholic extracts at a concentration representing 20 mg. per L. No crude sample has ever been obtained which is more pungent. Consequently, it is recommended that 20 mg. per L. be specified as the standard of pungency for U. S. Pharmacopœia capsicum.

#### BIOASSAY OF OLEORESIN OF CAPSICUM.

The U. S. Pharmacopœia X specifies that 0.2 Gm. of oleoresin of capsicum shows the same pungency as 1 Gm. of the crude drug. Since the specific gravity at 25° C. is about 0.925 (3) the desired volume of oleoresin was measured in a 1-cc. Mohr pipette. A series of oleoresins were obtained which had been manufactured by the usual commercial procedure and stored in 1-drachm bottles in the dark at room temperature. A series of commercial oleoresins were purchased on the open market under the specification that they should conform with U. S. Pharmacopœia X requirements. Oleoresins of several non-official varieties were prepared by C. Jelleff Carr following the method outlined in U. S. Pharmacopœia X. The pungency of these samples is reported in Table III.

TABLE III.  
PUNGENCY OF OLEORESINS OF CAPSICUM.

PA no.	Product.	Minimum effective concentration, mg. per L.	Remarks.
680	Stock sample	3.5	4 mo. old when assayed
674	Stock sample	3.5	5
679	Stock sample	3.0	6
678	Stock sample	3.5	9
677	Stock sample	3.5	11
676	Stock sample	3.5	24
675	Stock sample	3.5	27
686	Stock sample	3.5	18 $\frac{1}{2}$ years
670	Commercial sample No. 1	7.0	U. S. P. VIII
671	Commercial sample No. 2	10.0	U. S. P. VIII
672	Commercial sample No. 3	3.5	
673	Commercial sample No. 4	5.0	U. S. P. X
690	Japan chillies	20.0	
723	Louisiana sports	12.0	

According to manufacturing records, the yield of oleoresin PA No. 679 was about 20% lower than the yields of the other samples. It is interesting to note that this sample is about 20% stronger than the others. The fact that the same degree of pungency was noted in all of the stock samples, even Sample PA No. 686 which was 18 $\frac{1}{2}$  years old, demonstrates that the pungent principle does not deteriorate with age.

The variability of commercial samples is remarkable. Commercial samples Nos. 1 and 2 were labeled "U. S. P. VIII," in which acetone is specified as the solvent instead of ether. The pungency of oleoresins extracted from the same lot of capsicum by various solvents is now under investigation. Results to date have not indicated that acetone is inferior to ether as a solvent. Oleoresins from the nonofficial varieties were much less pungent.

The U. S. Pharmacopœia requirement of standard pungency is 3.5 mg. per L. From the results obtained in this investigation no change in this standard seems warranted.

#### PUNGENCY OF TINCTURES AND FLUIDEXTRACTS.

One sample of tincture and one of fluidextract were tested. The minimum effective concentrations were found to be 0.25 and 0.025 cc. per L., respectively. An effort was made to determine the capsicum content of a fluidextract of capsicum and myrrh, but the myrrh disguised the characteristic pungency, making determination of the end-point difficult.

#### PUNGENCY OF CAPSICUM SEED.

It is believed that the fixed oil resides in the seed, the volatile oil and capsaicin in the tissues. If there is no pungency in the seed they would serve solely as diluents. Seed from Sierra Leone, *Annuum* and *Japan* chillies were carefully separated from the tissue by hand and extracted with alcohol or ether; 200, 100 and 1000 mg. per L., respectively, failed to show evidence of pungency. Since the official oleoresin had a pungency of 3.5 mg. per L. it is believed that any slight

pungency found was due to mechanically present capsicum tissue and that the seeds themselves are not pungent.

#### PUNGENCY OF CAPSAICIN.

Through the kindness of E. K. Nelson, a sample of chemically pure capsaicin was obtained for this investigation. It was dissolved in 95% alcohol to furnish a stock solution containing one mg. per cc. This solution was stored in a glass-stoppered flask and small portions removed and diluted with 10% sucrose solution immediately before tasting. In each experiment several dilutions of capsaicin were prepared. Five cc. of the weakest solution was given to each member of the test group first. In case a definite pungency was reported no further tests were made for ten to fifteen minutes. In case no pungency was detected each tester rinsed the mouth, swallowed about 15 cc. of water, and a stronger concentration was administered. This was repeated until a definite reaction was obtained. Thereafter, several further tests were conducted in which the tester did not know the relative strengths of the solutions given. This was done in order to prevent bias.

Tests have been made upon a total of ninety-four different individuals. Some of this group were pharmacy students from two schools of pharmacy, others were nurses from two schools of nursing. The remainder of the group consisted of chemists, clerks, stenographers and factory employees. Some of the group had had a long experience in drug manufacture whereas others were inexperienced. By administering a non-pungent solution first the lower limit of detectile pungency was sought without fatiguing the taste perceptions.

The degree of response was estimated. No detectible pungency was reported as zero. A just distinctly perceptible pungency was reported as one plus. As the intensity of pungency increased the reactions were grouped as two plus, three plus and four plus which was taken as the maximal effect. The number of individuals giving different degrees of reaction to various doses are recorded in Table IV.

TABLE IV.  
PUNGENCY OF CAPSAICIN TO INDIVIDUALS.

Degree of pungency.	Concentration of capsaicin—mg. per L.									
	0.075	0.10	0.125	0.13	0.16	0.175	0.25	1.0	1.25	2.5
0	21	47	3	0	0	0	1	..	..	..
1 plus	..	47	12	2	2	1	5	3	1	..
2 plus	..	..	1	..	..	1	8	5	..	3
3 plus	..	..	..	..	..	..	2	5	..	1
4 plus	..	..	..	..	..	..	..	..	..	6
Total	21	94	16	2	2	2	16	13	1	10

The results given in Table IV show that there is a great variation in relative susceptibility. In no instance did a concentration of 0.075 mg. per L. cause a definite reaction for pungency. At 0.10 mg. per L. just one-half of the test subjects gave a positive response. It is estimated that 0.1125 mg. per L. will cause a positive reaction in 2 of 3 individuals. A concentration of 0.25 mg. per L. failed to cause any perception of pungency in only one instance. With the exception of this particular individual a concentration of 0.13 mg. per L. or above always



caused a reaction of one plus or more. The results obtained as a single plus reaction in terms of per cent of individuals tested is shown in Fig. 1. The results are approximately in agreement with a straight line having equation  $Y$  equals  $(X - 0.075)/(0.00055)$ , in which  $X$  represents the concentration of capsaicin in mg. per L. and  $Y$  the percentage of one-plus reactions to be expected. An increase

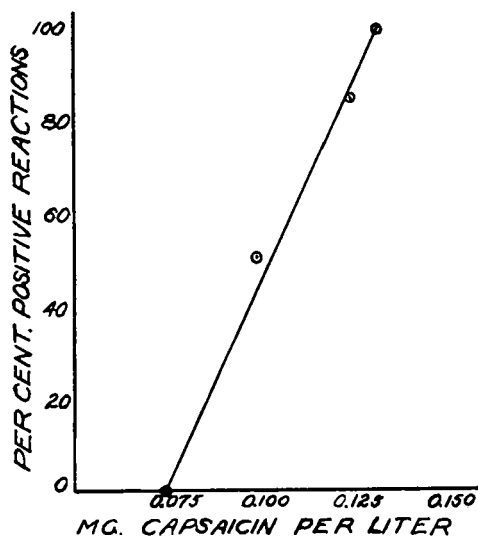


Fig. 1.

of 0.011 mg. per L. would cause an increase of 20% in positive reactions.

When the correlation between the concentration of capsaicin and the percentage of positive reactions is determined by the usual statistical methods (4), the correlation coefficient,  $r$ , is found to be plus 0.65, with a probable error of 0.04. These results indicate that there is a definite positive correlation between taste and dosage, and that this relationship is linear. Since this is true it becomes quite feasible to standardize individuals in terms of capsaicin, if it were available. A series of tests upon the same group of ten individuals over several months showed fairly uniform responses. There were occasional daily variations which at times

amounted to 10 to 20 per cent, but in general no change in threshold was noted.

No mention is made in the official method as modified regarding the interval which should elapse between successive tests. In three individuals showing a one-plus reaction to a concentration of 0.10 mg. of capsaicin per L., 5-cc. quantities of solution were swallowed at short intervals, as indicated in Table V. The degrees of response were found to be somewhat greater on the second than on the original test; thereafter the original degree of response was always produced.

TABLE V.

## REPETITIVE RESPONSE TO CAPSAICIN.

Seconds after first test.	SUBJECT.			Seconds after first test.	SUBJECT.		
	A.	B.	C.		A	B.	C.
0	1	1	1	75	1	.	.
15	2	2	.	90	1	.	.
30	1	1	.	100	1	.	.
45	1	1	.	120	1	1	.
60	1	1	2	135	.	1	.

A series of tests were conducted upon eight subjects showing a one-plus reaction to 0.10 mg. of capsaicin per L. in a 10 per cent sucrose solution. The same concentrations were administered in a 5 per cent, and in a 7.5 per cent sucrose solution. The apparent pungency was slightly weaker in the 5 and 7.5 per cent solutions as compared with the response in the 10 per cent solution, but eventually the same degrees of response were obtained.

## CONCLUSIONS.

1. Several modifications are suggested for the bioassay of capsicum and its products outlined in U. S. Pharmacopœia X.

2. A concentration of 0.10 mg. of capsaicin per L. in 10 per cent sucrose solution caused a definite sensation of pungency in the throats of half of ninety-four individuals tested, and 0.13 mg. per L. in the throats of all individuals.

3. Definite linear correlation has been found between concentration and percentage of positive reactions to capsaicin ( $r$  equals  $0.64 \pm 0.04$ ).

4. Using the modified method a number of official and unofficial capsicums, oleoresins and galenicals have been assayed. Great variability in commercial products has been found.

5. An oleoresin of capsicum 18 $\frac{1}{2}$  years old was as pungent as a freshly-prepared sample.

6. Need is pointed out for a definite standard by means of which individuals may standardize their pungency perceptions. Capsaicin would be very good but is not available in adequate quantities. Piperine has been found suitable and is recommended for this purpose.

7. As standards of pungency it is recommended that 20 mg. per L. of capsicum, or 3.5 mg. per L. of oleoresin of capsicum should produce the same degree of pungency as 16 mg. per L. of piperine, all solutions to represent alcoholic solutions and to be diluted with ten per cent sucrose solution.

8. Solutions differing by 10 per cent in pungency have been readily distinguished.

## BIBLIOGRAPHY.

- (1) Buchheim, *Vierteljahrschr. prakt. Pharm.*, 4 (1872), 507; through DuMez, page 121.
- (2) Bucholz, *Raschenb. Scheidkuenst. Apoth.*, 37 (1816), 1; through DuMez, page 122.
- (3) Andrew G. DuMez, "A Century of the United States Pharmacopœia, 1820-1920. I.—The Galenical Oleoresins," Inaug. Dissert. (1917), published as serial No. 980, *Bulletin of the University of Wisconsin*.
- (4) W. Palin Elderton, "Frequency-Curves and Correlation" (1906).
- (5) E. N. Gathercoal and R. E. Terry, "The Capsicum Monograph in U. S. P. X," *JOUR. A. PH. A.*, 10 (1921), 423-428.
- (6) W. Heubner, "Pharmacology of Irritating Substances," *Arch. exptl. Path. Pharmacol.*, 107 (1925), 129-154; through *Chem. Abstr.*, 19 (1925), 3325.
- (7) F. Victor Heydenreich, "Active Principle of Capsicum," *Am. J. Pharm.* (July 1858); through *Proc. A. Ph. A.*, 7 (1858), 58.
- (8) Landerer, *Vierteljahrschr. prakt. Pharm.*, 3 (1854), 34; through DuMez, page 122.
- (9) Charles H. LaWall, "A Method for the Detection of Small Quantities of Capsicum in Ginger Ale and Other Preparations of Ginger," *Am. J. Pharm.*, 81 (1909), 218-219.
- (10) A. Meyer, *Pharm. Zeit.* (1889), 130; through Tschirch, page 867.
- (11) Micko, *Zeitschr. Nahr. Genussm.*, 1 (1898), 818; 2 (1899), 411; through Nelson, page 1115.
- (12) Morbitz, *Pharm. Zeitschr. Russland.* (1897), 372; through DuMez, page 124.
- (13) E. K. Nelson, "Capsaicin, the Pungent Principle of Capsicum and the Detection of Capsicum," *Ind. & Eng. Chem.*, 2 (1910), 419-421.
- (14) *Ibid.*, "The Constitution of Capsaicin, the Pungent Principle of Capsicum," *J. Am. Chem. Soc.*, 41 (1919), 1115-1121.
- (15) *Ibid.*, "The Constitution of Capsaicin, the Pungent Principle of Capsicum. II," *J. Am. Chem. Soc.*, 42 (1920), 597-599.
- (16) E. K. Nelson and L. E. Dawson, "Constitution of Capsaicin, the Pungent Principle of Capsicum. III," *Ibid.*, 45 (1923), 2179-2181.

(17) Erwin Ott and Kurt Zimmermann, "Natural and Artificial Pepper Substances and the Relation between Chemical Constitution and Pepper Taste," *Ann.*, 425 (1921), 314-337; through *Chem. Abstr.*, 16 (1922), 1229.

(18) Erwin Ott and Fritz Eichler, "Natural and Artificial Pepper Substances. II. The Chavicin of Pepper Resin, the Especially Active Constituent of Black Pepper," *Ber.*, 55B (1922), 2653-2663; through *Chem. Abstr.*, 17 (1923), 1024.

(19) H. Rheinboldt, "The Pepper Taste of Piperine," *Ibid.*, 56B (1923), 1228-1229; through *Chem. Abstr.*, 17 (1923), 3189.

(20) C. Riccomanni, "Relations between Chemical Constitution and Taste," *Atti. accad. Lincei*, (5) 33 (1924), 145-158; through *Chem. Abstr.*, 18 (1924), 2900.

(21) W. L. Scoville, "Note on Capsicums," *Jour. A. Ph. A.*, 1 (1912), 453-454.

(22) W. L. Scoville, personal communication.

(23) Torald Sollmann, "A Manual of Pharmacology," 3rd Edition (1927), 211.

(24) Z. Stary, "Stimulation of Heat Nerves by Pharmaceutical Substances," *Arch. expll. Path. Pharmacol.*, 105 (1925), 76-87; through *Chem. Abstr.*, 19 (1925), 1602.

(25) H. Staudinger and H. Schneider, "Relation between Chemical Constitution and Pepper Taste," *Ber.*, 56B (1923), 699-711; through *Chem. Abstr.*, 17 (1923), 2581.

(26) H. Staudinger and F. Müller, "II Aliphatic-Aromatic Acid Piperides," *Ber.*, 56B (1923), 711-715; through *Chem. Abstr.*, 17 (1923), 2582.

(27) Strohmer, *Prager Rundsch.* (1884), 643; through Tschirch, page 867.

(28) Thresh, *Pharm. J. and Trans.* (3), 7 (1876-1877), 21, 259, 473; 8 (1877-1878), 187; through Nelson, page 1115.

(29) A. Tschirch, "Fructus Capsici," *Hand. der Pharmakog.*, 2nd Edition, 3 (1925), 867-878.

(30) John C. Umney, "What Is Capsicin?" *Pharm. J.*, 91 (1914), 594.

(31) "The Pharmacopœia of the United States of America. Tenth Decennial Revision," (1925), page 97.

(32) Elmer H. Wirth and E. N. Gathercoal, "Report of the Scoville Organoleptic Method for the Valuation of Capsicum," *Jour. A. Ph. A.*, 13 (1924), 217-219.

(33) R. Wasicky and F. Klein, "Über die Wertbestimmung von Capsicum," *Festschrift für A. Tschirch* (1926), 357-361.

#### ABSTRACT OF DISCUSSION.

W. L. Scoville said that Dr. Munch had added valuable information relative to the test. He asked whether Dr. Munch considered the reaction on the tongue as well as on the throat. He also asked whether piperine gave the same pungency on the throat as on the tongue. He had found variation in individuals not only personally, but at different times; he had tried the test on five different men, when they agree no further test is made; but if not, the test is tried on others. He had noticed that his own sensitiveness varied. If the test sample is quickly swallowed the results are not satisfactory. Referring to the skin test, he said that the response comes slowly and the individual, if busy with work, is apt to overlook the reaction. Sometimes the reaction comes and goes quickly. As a scientific test he did not consider the test as satisfactory as some others, but if practical application could be made of the test there was no reason for not making use of it.

James C. Munch replied he was endeavoring to make the method more quantitative so as to employ it in research work on capsicum, echinacea and other drugs. If cantharides is being tested there is no forgetting a definite amount on the skin will give a definite reaction.

He had been taking a reaction obtained on the throat within one minute as positive and ignoring the effects on the tongue; responses on the tongue result from stimulation of another nerve than that of the throat. He said that a man in the laboratory with him required ten times as much capsicum as he did and he also required ten times as much piperine. He recommended piperine or capsaicin for standardizing the individual.

In applying the test, 5 cc. of it, in five seconds, are allowed to trickle down the throat at a moderately slow rate.