This may have been due to vasodilation and increased blood supply to the glands.

On the other hand, irreversible sudomotor paralysis consistently followed injection of atropine. It is thus concluded, in agreement with Dale and Feldberg, that the sudomotor innervation in the cat is entirely cholinergic. Either there exists a marked species difference in cat and man in this respect, or the qualitative methods of observing sweating are misleading.

*Summary.* The effect of autonomic blocking agents on sweat gland response to single shock excitation of the sympathetic chain was studied in cats by a quantitative and objective method. Dibenamine, an adrenolytic and sympatholytic agent, failed to block sweat secretion whereas atropine consistently produced complete and irreversible sudomotor paralysis. It is concluded that the sudomotor innervation in cat is entirely cholinergic and possesses no adrenergic component, as reported in man.

## 16945

## Stable, Reduced, Desiccated Streptolysin "O."*

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A technic has been described[1] for the determination of the antistreptolysin "O" titer of serum which involved the employment of a concentrated lysin, diluted and reduced to the active form with a solution of cysteine hydrochloride at the time of use. This method has proved to be very satisfactory. Large amounts of the lysin have been prepared not oftener than every 12 months and deterioration during storage at circa 2°C has rarely been observed. A stable material not requiring refrigeration nor reduction at the time of use would be most desirable. This report describes a procedure for the preparation of a desiccated, reduced streptolysin "O" which, upon reconstitution with distilled water, is immediately ready for use.

*Streptolysin.* Concentrated streptolysin "O", prepared exactly as described in the paper mentioned above, is used.[1] Complete removal of sulphate ions is determined after dialysis by the addition of a drop of 25 percent barium chloride solution to a small amount of the concentrated material. A precipitate forms after dialysis and the addition of sodium chloride. It should be removed by centrifugation.

*Cysteine Hydrochloride.* The dry salt is required.

*Concentrated Buffer.* A concentrated (4 times normal) buffered saline solution is prepared according to the following formula.

| | g |
|---|---|
| NaCl | 68.0 |
| $KH_2PO_4$ | 45.2 |
| $Na_2HPO_4 \cdot 12\ H_2O$ | 74.6 |
| Distilled water to make 4 liters. | |

After the salts have been dissolved the acidity is checked with a glass electrode pH meter and adjusted to final pH of 6.5. The solution need not be sterilized.

*Buffered Solvent for Cysteine.* Seven and two-tenths grams of solid NaOH is added to one liter of normal buffered saline (1 part of concentrated buffer saline diluted with 3 parts of distilled water). At the time of reduction and dehydration an M/5 solution of cysteine hydrochloride is prepared by dissolving 3.0 g of the dry salt in 100 cc of this alkaline buffer. Final pH should be 6.5 to 7.2. The solution need not be sterilized.

* This investigation was conducted under the auspices of the Commission on Acute Respiratory Diseases, Army Epidemiological Board, Office of the Surgeon General, United States Army, Washington, D.C.

1 Rantz, L. A., and Randall, E., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 22.

TABLE I.

| | Volume of reagents | | | |
|---|---|---|---|---|
| | Lot 1 | | Lot 2 | |
| | For 1 bottle, ml | For 100 bottles, ml | For 1 bottle, ml | For 100 bottles, ml |
| Vol. of concentrated lysin containing 40 combining units | .875 | 87.5 | 1.5 | 150.0 |
| Vol. of M/5 cysteine | 2.5 | 250.0 | 2.5 | 250.0 |
| Vol. of normal buffer saline | (16.62) | | (16.0) | |
| Vol. of conc. buffer saline | 4.15 | 415.0 | 4.0 | 400.0 |
| Total to be added to each bottle before lyophilization | 7.52 | 7.52 | 8.0 | 8.0 |

*Method.* The object of the method is to have the lysin, salts and reducing agent combined in the proper proportions and in a volume conveniently small for lyophilization. Each finished bottle of dehydrated material contains exactly 40 combining units of streptolysin, the residue from 2.5 ml of M/5 cysteine solution and sufficient buffer salts so that the whole will be isotonic when reconstituted with 20 cc of distilled water. The following calculation is made: 20 ml less the volume of lysin containing 40 combining units plus 2.5 cc of M/5 cysteine equals the volume of normal buffer required. This value, divided by 4, gives the amount of concentrated buffer per bottle. The sum of the volumes of lysin containing 40 combining units, of M/5 cysteine, and of concentrated buffer saline is the amount of reduced buffered material to be transferred to each bottle before dehydration. The examples shown in Table I are illustrative.

The concentrated lysin, M/5 cysteine solution, and concentrated buffer are appropriately measured and mixed after complete preparations have been made for freezing and drying. The solution is dispensed with an accurate automatic pipette into standard 20 cc vials with an inside neck diameter of 12 mm. The maximum time which may elapse after reduction and before freezing is not known but 500 to 1,000 bottles may safely and satisfactorily be handled.

The filled bottles are vertically frozen at once in a shallow bath of methylcellosolve containing large amounts of dry ice, and desiccated from the frozen state in a commercial dehydrating apparatus at a pressure of 300 microns of mercury for 42 hours. The final moisture content is circa 2.5%. The vacuum is broken with sterile dry air and the bottles closed with a rubber flange stopper in a dry sterile stoppering cabinet and sealed with standard aluminum seals.†

*The Test.* One bottle of dehydrated, reduced lysin is reconstituted with 20 ml of distilled water. It dissolves at once to form a water clear solution and is ready for use. One combining unit is contained in 0.5 ml. The material may be used in any of the technics for determination of the antistreptolysin titer of serum. The dilution system previously described has proved to be very satisfactory.[1]

*Stability.* The dehydrated reduced lysin has been stored at 37°C, room temperature, and at 2°C for 18 months. Serial tests have been made and no loss of potency or alteration in the characteristics of the product has been noted. After reconstitution the material is highly unstable and should be used within 2 hours.

*Discussion and Summary.* A method is described for the preparation of a very stable, desiccated, reduced streptolysin "O". The material, when reconstituted with distilled water, is suitable for use in the determination of the antistreptolysin "O" titer of human serum. The availability of this material

† The cooperation of the Cutter Laboratories is gratefully acknowledged. Processing has been done in the plant of this company. Mr. B. E. Emery of that organization may be consulted for additional technical details.

greatly simplifies this procedure since it is always immediately ready for use without the addition of the reducing agent. In addition, it may be shipped and stored conveniently since refrigeration is not required. Its production on a large scale or commercial basis would not prove difficult if a sufficient need for this material should arise.

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## Influence of Heating on the Liberation of Certain Amino Acids from Whole Soybeans.*

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The effect of heating on the nutritive value of soybeans has been studied by a number of investigators. A review of such studies was made recently by Evans and McGinnis.[1] They further showed that the nutritive value of soybean proteins as determined by gain in weight per gram of protein consumed by chicks was increased when the soybean oil meal was autoclaved at 100°C, 110°C or 120°C for 30 minutes but to a smaller degree when it was autoclaved at 130°C for 30 or 60 minutes. The changes in nutritive value were found to parallel the availability of the methionine and cystine of the soybean.

Clandinin *et al.*[2] reported that heating solvent-extracted soybean flakes in an autoclave at 15 lb pressure for 4 minutes resulted in a meal of high nutritive value for the chick; however, when the heating was prolonged to 4 hours a decrease in the nutritive value was observed. These changes were shown by Riesen *et al.*[3] to be related to an increase in the amount of each of the essential amino acids liberated by pancreatic hydrolysis with the short period of heating and a decrease with the prolonged heat treatment.

More recently Klose *et al.*[4] reported that treatment of raw soybeans with steam at 15 lb pressure gave a product of maximum nutritional value for the rat after 10 to 15 minutes while heating beyond this time resulted in a gradual decrease owing to a resultant deficiency of methionine, lysine and leucine. Steam treatment at about atmospheric pressure for 30 minutes gave a product showing a slightly higher maximum and there was little decrease in nutritive value on prolonged heating.

Soybeans have been used in the Chinese diet for centuries and one of the methods of cooking is to boil in water for 2 to 3 hours until the beans are quite soft. The present investigation was designed to determine the effect of boiling soybeans in water at different periods of time on the liberation of certain essential amino acids by acid and by enzymatic digestion.

*Methods.* The soybeans used were recently harvested, dried seeds of the Hawkeye

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1 Evans, R. J., and McGinnis, J., *J. Nutrition*, 1946, **31**, 449.

2 Clandinin, D. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *Poultry Sci.*, 1947, **24**, 150.

3 Riesen, W. H., Clandinin, D. R., Elvehjem, C. A., and Cravens, W. W., *J. Biol. Chem.*, 1947, **167**, 143.

4 Klose, A. A., Hill, B., and Fevold, H. L., *Food Tech.*, 1948, **2**, 201.