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Purification of Paralytic Shellfish Poison by Filtration Through Active Charcoal.

WILLIAM M. BENDIEN AND HERMANN SOMMER. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, University of California, San Francisco.

In previous attempts to isolate the paralytic shellfish poison[1-5] various adsorbents and base exchange materials proved helpful for the purification of this highly potent organic base. Mueller[3] made use of Norit to remove pigments from acid alcoholic solutions of the poison; under these conditions the active substance is found quantitatively in the filtrate. Monnier[4] found that Norit adsorbs the poison completely from a neutral aqueous solution. Difficulties in eluting the substance quantitatively with acid indicated that this adsorption was not a simple base-exchange reaction. More detailed investigations now reveal that, besides the base exchange, a chromatographic type of adsorption is involved which can be utilized to effect a considerable purification of the poison, as the following examples show.

A poison solution was first used which had been partly purified by Monnier[4] by filtration through permutit and consequently contained mostly salts of strongly basic substances. The solution was adjusted to 150 lethal mouse units per cc and an acidity of 1 n HCl. When such a solution is filtered through a column of purified Norit A the filtrate is practically free of poison. Nor does the washing with a few cc of water remove appreciable amounts of the active substance. On further washing with water, however, practically all of the poison is eluted, in a greatly purified form. Traces of acid present obviously prevent a base exchange adsorption on the charcoal. A typical example is given in Table I.

Crude mussel extracts can also be purified by this procedure, provided they contain 150 MU per cc. The mussel livers are extracted with acid methyl alcohol (0.04 n HCl). Filtration through Norit now only removes the pigments and colloids. The resulting

1 Meyer, K. F., Sommer, H., and Schoenholz, P., *J. Prev. Med.*, 1928, **2**, 365.

2 Prinzmetal, M., Sommer, H., and Leake, C. D., *J. Pharmacol.*, 1932, **46**, 63.

3 Mueller, H., *J. Pharmacol.*, 1935, **53**, 67.

4 Monnier, R. P., Thesis, Eidgen. Techn. Hoehsch., Zürich, 1938.

5 Sommer and Monnier, unpublished data.

TABLE I.
Purification of Shellfish Poison by Filtration through Norit. Dimensions of column: 80x11 mm; rate of filtration approx. ½ cc per min.

	Vol. cc	Acidity, n	Concentration, MU* per cc	Amount, MU	Purity, γ per MU	Yield, %
Starting material	20	1.0	150	3000	25	100
Filtrate	20		5	100		3
Wash water	5	0.5	5	25		1
Eluate I (water)	10	0.1	265	2650	3.3	93
Eluate II "	5		30	150		

*MU—Lethal mouse units.

colorless solution is evaporated to dryness, the residue taken up in aqueous HCl, 1 n and purified as above. With mussels in which the poison content is high compared to the pigments and colloids the preliminary purification can be omitted. By a single filtration of the acid aqueous solution the pigments are removed by irreversible adsorption, the salts and other inert electrolytes are discarded in the filtrate and the poison is concentrated in the eluate. By repetition of the procedure under identical conditions an over-all yield of 70% and a 10-fold purification (from 50 γ to 4.7 γ per MU) are obtained. The saving of time and precious material by this method is evident as compared with other procedures.

The following analytical tests were made with poison samples of a potency of 3.5 γ per MU, which are comparable to the best products obtained by Monnier.[4]

Fluorescence. The products obtained showed no fluorescence in the ultraviolet, in contrast to some of the crude poison preparations. On detoxification with boiling alkali the fluorescent substance reappears, indicating that this is an atoxic transformation product of the poison.

Ash. Since the inorganic constituents are much less retarded by the filtration than the toxic substance the resulting products are low in ash. In 2 cases 2.4 ± 0.6% and 0.0 ± 0.5% ash were obtained.

Nitrogen and Chlorine. Determinations of nitrogen (by micro-Kjeldahl) and chlorine in different samples showed from 7.0 to 7.5% and 17.5 to 19.8% respectively, giving in all cases one chlorine ion for each atom of nitrogen. This indicates that all the nitrogen present is of basic nature.

Test with Nessler's Reagent. Of the various reagents tested only Nessler's solution was found to give a positive test. A greenish yellow coloration and turbidity are obtained, resembling strongly the test obtained with aliphatic diamines. Further purification ex-

periments will have to show whether the test is specific for the poison or due to an accompanying impurity.

Summary. A simple and efficient method for the purification of shellfish poison consists in differential retardation when the poison is filtered through a charcoal column. The resulting products are low in ash, have lost their fluorescence, have an equal number of Cl and N atoms and give a characteristic Nessler reaction.

After completion of this work the article by Tiselius (*Science*, 1941, **94**, 145) came to our attention in which the author demonstrated the differential retardation of various salts, sugars, amino acids and peptides through active carbon and other adsorbents and proposes the procedure as a method of analysis of wide application. The separation of the colorless substances is, in this case, made visible by the Schlieren method.

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Production of Anemia by Tetanus Toxin.

HANNAH FARKAS AND I. J. KLIGLER.

From the Department of Hygiene and Bacteriology, Hebrew University, Jerusalem.

Until the recent extensive studies by Abel[1] it was generally accepted that tetanus toxin reaches the central nervous system by way of the nerve tracts. This view was based largely on the experimental work of Marie, Marie and Morax and others.[2] However, the experiments of Abel and his associates[1] showed that the toxin was distributed through the body by way of the lymphatic and blood systems, the specific effect of the toxin being produced on the nerve tissue with which it came in contact. Even prior to Abel's work, Van den Reis[3] according to Weinberg suggested that tetanus toxin caused a secondary anemia.

The object of the experiments reported below was to study the effect of tetanus toxin on the blood. The experiments were made on rabbits which in contrast with guinea pigs, rats and mice gave a constant red cell count. Individual normal rabbits examined regularly during a period of one month showed only slight variations in total

[1] Abel, J. J., *Science*, 1934, **79**, 63, 121; *Bull. Johns Hopk. Hosp.*, 1935a, **56**, 84, 317; 1935, **57**, 343.

[2] Marie, A., *Ann. Inst. Pasteur*, 1897, **11**, 591; 1898, **12**, 91; Marie, A., and Morax, V., *Ann. Inst. Pasteur*, 1902, **16**, 818; Meyer, H., and Ransom, F., *Arch. Path. Pharm.*, 1903, **49**, 369.

[3] Van den Reis, *K. W.*, 1922, **1**, 950.