

the variable quantities of the more complex amino-acids, which do not give a color proportional to their nitrogen content. It appears that easily hydrolyzed peptides are responsible for a large percent of the undetermined rest nitrogen.

Non-protein Nitrogen Determinations per 100 cc. of Whole Blood (Human).

No.	Total Non- Protein N	Urea N	Preformed Creatinin N	Total Creatinin N	Uric Acid N	Amino Acid N	Und ¹ - ter'd Rest N	Pep- tide N	Peptide N % of Und ¹ ter'd Rest N
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	
1	29	13	0.5	1.6	0.9	8.7	4.8	4.3	90
2	38	17	0.8	1.5	0.84	7.0	11.8	11.0	93
3	45	17.8	0.8	2.0	0.88	8.7	15.6	11.0	70
4	42	11				8.0	23.0	20.0	
5	40	17				8.3	14.7	13.3	
6	40	13.8				7.8	18.4	13.0	
7	43	16.5				7.8	19.0	15.0	
8	36	17.6				9.1	9.3	5.2	
9	41.5	14				8.7	18.8	11.3	
10	142	108	1.8	4.6	1.7	8.7	19.0	13.8	72
11	114	91				8.0	15.0	7.0	
12	115	90			1.6	7.0	16.4	14.0	
13	150	114	3.0		2.0	7.7	23.3	20.0	
14	154	120		3.2	2.6	7.3	21.0	16.0	76

95 (2618)

The effect of sodium ricinoleate upon bacterial toxins, and the value of soap-toxin mixtures as antigens.

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The present study is a continuation of the work published by Larson and Nelson¹ on the effect of castor oil soap upon bacterial toxins. It was shown that sodium ricinoleate possessed the property of detoxifying soluble toxins as well as endotoxins.

In the present paper an attempt is made to analyze the mechanisms of the detoxifying action of soaps, and further, to study the antigenic properties of toxins which have been thus detoxified.

¹ PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 278.

The work was begun by subjecting diphtheritic toxin and tetanus toxin to the action of the sodium salts of a large number of fatty acids as: ricinoleic, oleic, stearic, palmitic, myristic and lauric acids, and two acids with uneven numbered carbon chains, ennenic and hendecenoic acids. Our procedure was to mix the toxin with an equal volume of a one per cent soap solution except in those cases where a one percent solution of soap gels, in which cases weaker solutions were used. It was found that the soaps of oleic, stearic and palmitic acids had no detoxifying action whatever on bacterial toxins. The soaps of lauric, myristic, ennenic and hendecenoic acids possess some detoxifying action upon toxins, but do not compare with sodium ricinoleate in detoxifying properties.

The soaps which detoxify bacterial toxins, differ in physical properties from other soaps, in that they form clear, or nearly clear, solutions in physiological NaCl solution, and dialyze readily through hardened collodium sacs. The detoxifying soaps depress the liquid air surface tension more than do the other soaps of equivalent concentrations used in our experiments.

Early in our work we were impressed by the fact that the detoxifying power of a soap seemed to be in proportion to its solubility in water and inversely with its tendency to gel. It was found that a slightly turbid solution of sodium ricinoleate would not detoxify as effectively as a perfectly clear solution even though the surface tension of the air liquid phase was unchanged. The addition of sodium oleate or sodium stearate to sodium ricinoleate, deprived the latter of its power to detoxify. Colloidal calcium soap, starch, blood serum and nutrient broth have a similar effect. The explanation of this observation may be found in the fact that soap and other surface tension depressants are adsorbed into the surfaces of the colloidal aggregates and other suspended particles in the fluid, and the soap therefore is not free to act upon the toxin molecule. This would lead to the further assumption that the detoxifying action of soap upon bacterial toxin is an adsorption phenomenon. The soap probably dissolves into the surface of the toxin molecule, thus imprisoning it so that it is not free to react with the tissues in the usual manner.

The question naturally arises as to whether the toxin is actually destroyed or merely held imprisoned for a time. In order to shed

some light on this point, very large doses of toxin-soap mixtures were injected into animals. In working with tetanus toxin, we arbitrarily chose as a unit the amount of toxin that would kill a guinea pig, of 400 grams in 24 hours. This was one-half cc. in volume of the particular toxin used. It was found that 0.2 cc. of a one percent solution of sodium ricinoleate was sufficient to completely detoxify this amount of toxin as far as could be determined by guinea pig inoculation. If this amount of soap solution destroys the toxin completely, the guinea pig should be able to withstand multiples of these amounts of soap and toxin without developing symptoms of tetanus. However, when multiples of these amounts were injected, it was found that the animals developed tetanus in periods of time varying with the amount of toxin used. This led us to the conclusion that the toxin molecule is not actually destroyed, but merely imprisoned for a time. The toxin is apparently slowly released at a rate which has not been definitely worked out. When the dose of toxin has not been too large, the release is so slow as not to cause symptoms of tetanus. When the amount of soap-toxin given is greatly increased, the liberation of toxin in a given time is naturally larger, and the animal develops tetanus. The same law holds true of diphtheritic toxin.

The fact that the soap does not change the properties of the toxin molecule is supported by the further observation that both diphtheritic and tetanus toxin which have been detoxified with soap are excellent antigens, as both rabbits and guinea pigs may readily be immunized by treatment with toxin-soap solutions. The serum of rabbits has been found to contain a high titre of antibodies against tetanus toxin after having received four injections over a period of five weeks. Likewise it has been possible to immunize guinea pigs against both diphtheritic and tetanus toxin by this method. We believe that this method of immunization is practical in immunizing humans against diphtheritic toxin. This work is now under way.