ON THE ANTIGENICITY OF DIPHTHERIA TOXOID.
PART II. PRELIMINARY REPORT ON THE IMMUNIZING
POTENCY OF DIPHTHERIA TOXOID FRACTIONS ISOLATED
AT LOWER AMMONIUM SULFATE CONCENTRATION

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INTRODUCTION

In the preceding paper¹⁾, Kurokawa and others scrutinized several methods for the measurement of immunizing potency of diphtheria toxoid, which have generally been used hitherto, and devised one method or two for precise measurement which suit for routine use in laboratories, because of their comparative accuracy and less expenses. On the basis of the results thus acquired, Kurokawa and others are performing the analytical studies on the factors participating in the immunizing potency of diphtheria toxoid.

In the studies on the methods for the measurement of immunizing potency in the preceding paper, several kinds of highly purified toxoid were used as the experimental materials. These highly purified toxoids were fractions precipitated between 30 per cent and 60 per cent saturation with ammonium sulfate. These fractions did not seem to denature during the process of purification, but it appeared to be so sensitive in vivo that the immunizing potency was markedly affected by the diluent used for injection.

While the fraction precipitated at 30 per cent saturation with ammonium sulfate, designated as "30% fraction" in this paper, is generally discarded in the process of purification, the nature of the "30% fraction" in respect to the immunizing potency seems not to have been reported yet.

This paper concerned with some properties of fractions of this type isolated at low ammonium sulfate concentrations.

EXPERIMENT

A material, AFr₃₀ II, isolated at the 30 per cent saturation with ammonium sulfate during the course of preparation of highly purified toxoid

thus obtained was larger than that of AFr₃₀₋₆₀ II and Lf was equal to the sum of each fraction and the immunizing potency did not seem to fluctuate from that of AFr₃₀₋₆₀ II (group C, Table 1). In other words, it seemed to be more rational to consider that the toxoid itself contained in AFr₃₀ II is different in respect to the immunizing potency from the toxoid contained in AFr₃₀₋₆₀ II, rather than that there are something else, but not toxoid, affecting the immunizing potency of the toxoid contained in the fraction.

Immunizing potencies of AFr₃₀ II and AFr₃₀₋₆₀ II were compared again and the same result was obtained (groups D and E, Table 1). Immunizing potencies of other materials, AFr₂₅ IV, AFr₃₀₋₃₅ IV and AFr₃₅₋₆₀ IV, prepared from another crude toxoid, preparation method of which was described in Table 2, were compared and it was observed that the fractions precipitated at lower concentrations of ammonium sulfate had evidently lower potency (Table 2).

However, the means of antitoxin titer of groups D and E in Table 1 and of all groups in Table 2 were evidently lower than that of groups A, B and C and that observed in the similar experiments performed in our laboratory hitherto. The test toxin used in the measurement of antitoxin titer was the same and no significant variation of the test dose was observed throughout the whole experiments. And, in each animal, the relation between the antitoxin content of blood and the tolerance to toxin, subcutaneously (Table 1 and 2) or intracutaneously (Table 2) injected, coincided with the results described in the preceding paper.¹⁾ Therefore, unusually low titers of antitoxin above described do not seem to be due to procedure of titration. In this respect, it may be of special interest to describe that the experiments of groups A, B and C in Table 1, groups D and E in Table 1 and groups in Table 2 were performed during the period from the end of September to the end of October, during the period of the end of October to the end of November, and during the period of January and February respectively, and that the other experiments performed in the identical manner during the same period also showed similar tendency (Table 3). Though several reports that the antitoxin response of animals was influenced by season have been published,²⁾ this problem will be described in a separate paper. In this paper, only the results obtained during the same period were compared each other.

The ratios of total Lf of fraction precipitated at the 30 per cent saturation to total Lf recovered were found to range from less than 10 per

Table 2.

Immunizing potency of fractions isolated at low ammonium sulfate concentrations. (2)

Groups	Samples	Lf dosis inoc.	Kf	Antitoxin unit/ml 3)		Date of toxoid inoculation
				average titer	animals tested	
A	A _{Fr₂₅} IV	20	420+120(25)§	0.0004	(5) * **	17/Jan. 1951
B	A _{Fr₃₀₋₃₅} IV	20	145 (25)§	0.004	(6) *	
C	A _{Fr₃₅₋₆₀} IV	20	150 (15)§	0.012	(5) **	

1) Method of preparation as follows:

2) § indicates to have been performed by means of the blended method.
3) ** indicates that difference of average titers is significant at the 5% level of risk between A and B, 1% between A and C, respectively.

cent to more than 50 per cent for various kinds of toxoid preparation, crude or partially purified, manufactured by various plants in our country. The conditions increasing this ratio during the process of purification or detoxification and the role played by these fractions in immunizing potency of toxoid are still under investigations. As an instance showing the relation between the immunizing potency and the ratio of total Lf of "30% fraction" to total Lf recovered, one of the experiments performed in respect to this problem was shown in Table 3, by which it seems, in most cases at least, that there exists a tendency which the more the "30% fraction" is contained in toxoid, the less the immunizing potency of toxoid becomes. Moreover, from Table 3, it seems that the condition causing the Kf increase may be another factor responsible for the discrepancy between the Lf value and the immunizing potency *in vivo*.

Table 3.

Immunizing potency of several toxoid preparations.

Toxoids	Lf dosis injec.	Kf	% of total Lf of fractions to total Lf recovered		Antitoxin u/ml average animals titer tested	Survival rate (10 MLD)
			30% frac.	30-60% frac.		
C	13	60(36)	6	94	0.007 (7)	7/7
K	12	220 + 50 * (24)	9	91		4/9
O	16	130(33)	15	85		11/11
U	27	40(55)	17	83		7/9
R1	13	∞	17	83		3/7
D	15	90(30)	19	81	0.003 (8)	5/6
A	7.5	∞	20	80		0/9
F	18	190(37)	40	60	0.002 (9)	5/8
R2	13	130(26)	40	60		6/10
Q1	30	90(60)	50	50	0.001 (9)	2/7
I	11	450(22)	66	34		1/10
Q2	27	90(54)	68	32	0.001 (9)	1/8

* Flocculation appeared after it was left standing overnight in cold chamber.

The results described hitherto in this paper seem to give one of the bases for interpretation of nature of diphtheria toxoid in respect to immunizing potency or for selection of method of purification, and further, a part of discrepancy between the Lf value and the immunizing potency *in vivo* may be explained by them. It may be interesting to recall in this respect that Pappenheimer³⁾ and Agner⁴⁾ noted that diphtheria toxin

precipitated at low ammonium sulfate concentration was flocculated very slowly and possessed low toxicity per Lf unit.

SUMMARY

The immunizing potency of the fractions of diphtheria toxoid precipitated at lower ammonium sulfate concentrations were evidently lower than that of fractions precipitated at higher ammonium sulfate concentrations.

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