

Mirsky<sup>2</sup> while those in the sap were made by Dr. M. Dole.<sup>3</sup> I wish to thank them for their kindness and courtesy.

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## The Isoelectric Point of the Dick Toxin.

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In the course of experiments having for their purpose purification of Dick toxin it became desirable to ascertain the electrical charge carried by the toxic material. This point seemed worthy of investigation not only because of our own immediate needs for such information, but also because the electrical charge borne by a toxin is an important factor in filtration, adsorption, flocculation, etc.

The method described by Krueger, Ritter and Smith,<sup>1</sup> based upon electrophoresis of the test substance into agar and subsequent resuspension in some appropriate menstruum, was employed. Toxin\* of high titre was adjusted to the required pH value with N/10 NaOH or HCl, placed in the two cells of the apparatus and exposed to a current of 70-85 v. and 5-12 milliamps. for 20 hours. At the end of this time the agar cylinders from anode and cathode were removed and triturated with 10 cc. normal saline solution for 1 hour. The extracts so obtained were then made up in 1/100 dilutions with normal saline and injected in quantities of 0.1 cc. into the skin of individuals known to be Dick positive. Each test patient also received a control injection, *i. e.*, 0.1 cc. of a 1:10,000 dilution of toxin previously adjusted to the pH at which the test was run, maintained at this  $C_{H+}$  for the same length of time as the toxin in the apparatus and neutralized before injection. This injection served to detect inactivation of the toxin by acid or alkali and also furnished us with a criterion by which to read the other skin reactions. We found it necessary to employ in the cataphoresis cells a gel containing not more than 0.4% purified agar. Percentages higher than this did not permit the toxin to migrate.

<sup>2</sup> Mirsky, A. E., and Anson, M. L., *J. Biol. Chem.*, 1929, lxxxi, 581.

<sup>3</sup> MacInnes, D. A., and Dole, M., *J. Gen. Physiol.*, 1928-29, xii, 805.

<sup>1</sup> Krueger, A. P., Ritter, R. C., and Smith, S. P., *J. Exp. Med.*, 1929, (in press).

\* Dr. Gladys Dick very kindly supplied us with large quantities of toxin having a titre of 12,500 S.T.D.'s per cc.

TABLE I.  
*Electrophoresis of Dick Toxin in Broth.*

pH	Hours current passed	E.M.F.	Milliamps.	Skin reaction 0.1 cc. intracutaneously			Nitrogen content. Mg./cc. of agar extract	
				Toxin control 1/10,000 dilution	Anode sample 1/100 dilution of extract	Cathode sample 1/100 dilution of extract	Cathode sample	
							Anode sample	Cathode sample
5.0	21	75	5.5	(+)	(—)	(+)	0.066	0.174
6.0	20	80	5.0	(+)	(—)	(+)	0.068	0.134
6.5	20	75	9.0	(+)	(—)	(+)	0.096	0.128
7.0	20	82	8.0	(+)	(—)	(+)	0.087	0.172
7.0	20	85	12.0	(+)	(—)	(+)	0.107	0.138
7.5	21	80	6.7	(+)	(+)	(—)	0.076	0.202
7.5	20	76	8.0	(+)	(+)	(—)	0.081	0.210
8.0	20	75	9.0	(+)	(+)	(—)	0.173	0.098
8.0	21	80	7.6	(+)	(+)	(—)	0.165	0.110
9.0	20	80	7.0	(+)	(+)	(—)	0.144	0.081
10.0	20	85	8.0	(+)	(+)	(—)	0.173	0.106

The skin-test data presented in Table I indicate that the isoelectric point of Dick toxin lies between pH 7.0 and pH 7.5. Judging from the nitrogen content of the anode and cathode samples, however, the reversal in electrical charge occurs between pH 8.0 and pH 7.5. This anomaly is explained by the results of a series of identical experiments run with broth alone (Table II). Here it is apparent

TABLE II.  
*Electrophoresis of Broth without Toxin.*

pH	Hours current passed	E.M.F.	Milliamps.	Nitrogen content. Mg./cc. of agar extract.	
				Anode sample	Cathode sample
5.25	24	70	5.5	0.042	0.091
6.0	24	70	5.2	0.061	0.130
7.0	24	65	7.0	0.042	0.160
7.5	25	66	4.8	0.040	0.138
8.0	24	65	6.0	0.118	0.056
9.0	26	65	10.5	0.161	0.038

that the shift in nitrogen values takes place between pH 8.0 and pH 7.5 and it is probably the migration of non-toxic, nitrogen-containing compounds normally present in broth that causes the difference noted above. The control series in Table I is of interest in that it demonstrates the resistance of Dick toxin (in relatively high concentration) to changes in  $C_{H+}$  from pH 5.0 to pH 10.0.

Since we were not working with a pure toxin but with a toxin-broth mixture we have no reason to suppose that the determined isoelectric range is that of the toxin itself. There is the possibility that the toxin exists in physical or chemical combination with some broth constituent and that it is this aggregate which migrates in the electric field. Such a micella may well have quite a different isoelectric point from that of the pure toxin.

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### Antemortem Basopenia as an Index of Resistance.

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In a recent study of rabbit's blood<sup>1</sup> it was shown that the basophile level before inoculation seemed to bear some relation to the course of infection with *Treponema pallidum* and it was suggested

<sup>1</sup> Casey, A. E., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 670.