Submerged Culture Production of Diphtheria Toxin

It has been shown[1–3] that high-titre diphtheria toxin can be produced by growing the Park Williams No. 8 strain of *C. diphtheriae* in shaking flasks, and one of us[4] has reported the production of 100 Lf. u./ml. toxins in stirred and aerated cultures. Recently, using the latter technique, we have been able to prepare high-titre toxins (Lf. 150–200 u./ml.) by growing the organism for 48 hr. in 14-litre amounts of tryptic digest medium[1] in 20-litre 'Pyrex' flasks stirred by either a glass or a 'Birmabright' (a magnesium–aluminium alloy) stirrer with air bubbling through the cultures from an 'Aerox' filter (porosity *P* 28) at the rate of one-sixth litre of air per litre of medium per minute. Such culture filtrates contain 1,500–1,700 Lf./mgm. protein nitrogen and are, therefore, very suitable starting material for the further purification of the diphtheria toxin. Moreover, the antigen composition of the culture filtrates, concentrated by ultrafiltration, when examined by a gel diffusion test against antitoxin[5], was found to be as complex as in surface-culture filtrates; but the amounts of the contaminating antigens were less, thus explaining the higher purities obtained from submerged cultures.

Further experiments using glass-lined vessels stirred with aluminium 'vortex' stirrers of a type similar to that suggested by Chain[6], with air being passed over the surface of the medium and not through a bubbler or sparger ring, have also given as good results as those obtained with 'Pyrex' glass flasks. Experiments with culture vessels constructed of commercial aluminium have given quite high titre toxins, but such vessels show signs of attack by the culture on continued use, accompanied by a decrease in toxin production. This problem is now under investigation.

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[1] Linggood, F. V., and Fenton, E. L., *Brit. J. Exp. Path.*, **28**, 354 (1947).
[2] Mitsuhashi, S., Kurokawa, M., and Kojima, Y., *Jap. J. Exp. Med.*, **20**, 261 (1949).
[3] Howatt, F. F., and Reed, G. B., *Canadian J. Research*, **28**, 23 (1950).
[4] Linggood, F. V., VIth International Congress for Microbiology, Rome (1953).
[5] Pope, C. G., Stevens, M. F., Caspary, E. A., and Fenton, E. L., *Brit. J. Exp. Path.* **32**, 3, 246 (1951).
[6] Chain, E. B., Paladino, S., Callow, D. S., Ugolini, F., and Van Der Sluis, J., *Bull. World Health Org.*, **6**, 73 (1952).

Blood Platelets as Carriers of Adrenaline and Noradrenaline

It is now widely accepted that, provided the sympathetic innervation is intact, adrenaline is discharged continuously from the suprarenal medulla, even under basal conditions. Several authors[1] found a basal discharge of 0·03–0·06 μgm./kgm./min. from the two suprarenals of cats and dogs. According to our results[2], the adrenaline arteriovenous difference in man is about 0·5 μgm./l. of blood, which corresponds, as a first approximation, to a rate of utilization of about 0·03 μgm./kgm./min.—a value in good agreement with the established rate of adrenaline discharge.

An arteriovenous difference of 0·5 μgm./l. is compatible with the figures which we find for the concentration of adrenaline in venous plasma, namely, 1·0–1·6 μgm./l. of blood[3]. The validity of the method used has been checked by paper-chromatographic studies which showed that the material estimated could be recovered from the expected positions in yields approximating to those of model experiments, and that the fluorescence developed in the eluates had the expected spectral properties[2].

However, Holzbauer and Vogt[4], on the basis of a biological method, recently came to the conclusion that the adrenaline content of plasma is much lower. They also found that insulin injection increased the plasma adrenaline concentration, whereas we had found the opposite effect[5].

It occurred to us that these discrepancies might be explained, at least in part, by the different methods of blood collection. Holzbauer and Vogt, conforming to the exigencies of the biological method, took every precaution to prevent platelet breakdown, whereas in our method[6] the blood is collected in a hypertonic solution and in untreated glassware, conditions known to lead to the disintegration of the blood platelets. 209 ml. blood was collected in a silicone-coated cylinder containing 3,500 units of heparin dissolved in 1·5 ml. isotonic sodium chloride; 15 ml. was withdrawn and mixed with 5 ml. of a sodium fluoride – sodium thiosulphate solution[6] for estimation by method of Weil-Malherbe and Bone[3], the remainder centrifuged in silicone-coated tubes at ~ 600 *g* for 20 min. Plasma (72 ml.) was separated and half of it recentrifuged at ~ 3,000 *g* for 20 min. Both plasma fractions, and the residue from the second spin, were transferred to untreated glassware and mixed with the sodium fluoride – sodium thiosulphate solution and acetate buffer, prior to chromatography on alumina columns[6]. The eluates from the two plasma fractions were each divided into four parts: one part for estimation by the ethylenediamine method[3] and three parts for estimation according to Lund[7] (one part each for oxidation at *p*H 3·0, oxidation at *p*H 6·5 and for the blank).

It appears from the results shown in Tables 1 and 2 that the substances estimated as adrenaline and noradrenaline by our method are indeed associated, to a large extent, with the blood platelets. When platelet disintegration is avoided, about 70–80 per cent may be removed from plasma by centrifuging and a considerable part may be recovered from the platelet residue.

Table 1. Estimation of Adrenaline and Noradrenaline (μgm./l. blood) in 'Platelet-rich' and 'Platelet-poor' Plasma

	Assay by ethylenediamine method (ref. 3)		Assay by Lund's method (ref. 7)	
	Adren.	Noradren.	Adren.	Noradren.
Blood collected in $NaF–Na_2S_2O_3$ solution	1·46	3·70	1·56*	5·40*
Blood collected in heparin and silicon-ized glass:				
Plasma spun at ~600 *g*	1·47	4·25	2·37	2·96
Plasma spun at ~3,000 *g*	0·34	1·20	0·36	1·17
Residue from second spin	0·72	2·11		

* These values were determined on a different blood sample collected from the same subject some days previously.