According to the investigation of Cass *et al.*[3] guanethidine depletes the NE in heart and spleen of rabbits and cats without lowering NE in the brain. The cause of the contradiction in the aforesaid and present data may be due to the species difference. According to the above-mentioned authors the drug probably cannot penetrate the brain owing to its low solubility in lipoid. The present result, however, namely that it inhibits the effect of Amphetamine, indicates its penetration into the central nervous system.

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Separation of a transplantation antigen

(*Received* 6 *January* 1962; *accepted* 17 *January* 1962)

TRANSPLANTATION antigens have successively been assumed to be: deoxyribonucleoproteins,[1] mucopolysaccharides of the blood groups type,[2] and glycoproteins.[3] The controversy is due to the fact that the antigens have never been purified. The main hindrance to the purification of those substances has been the time-consuming test for antigenicity (*in vivo* test).[1] Recent work[4, 5] raised the possibility of detecting transplantation antigens by a serological method (*in vitro* test). Hereafter, *in vivo* activity will be referred to as sensitizing activity and *in vitro* activity as serological activity. As the identity of the agents in both tests has yet to be confirmed, the main fractions studied here have been checked for their sensitizing as well as for their serological activity.

Mice of the inbred strains A/Jax (H-2^a), C57BL/6J (H-2^b), CBA (H-2^k) and ASW (H-2^s) were used.

Extraction. Antigens have been extracted from spleen and thymus cells as previously described.[3] The insoluble residue after lyophilization was made up of proteins, lipids and carbohydrates. Its serological and sensitizing activity was high.

Solubilization. A very mild acid hydrolysis (HCl, pH 2, 50 °C, 4 hr) led to part of the residue being solubilized. Centrifugation (100,000 g, 1 hr) provided a supernatant which exhibited a high serological activity and a poor sensitizing activity. It was soluble in water and contained proteins, polypeptides, lipids and carbohydrates, among which free sialic acid.

Fractionation. The supernatant was separated by dialysis against distilled water (48 hr, Visking cellulose casing 8/32) into a serologically inactive dialysable fraction, and a serologically highly active non-dialysable fraction. The latter was obtained in the form of a very hygroscopic white powder (dry weight, 0·01 per cent of the wet weight of fresh organs) giving a perfectly clear solution in water and

* Details will be published elsewhere.

salt solutions of low ionic strength. Paper electrophoresis (Whatman 3MM and Hurlbut 934 A.H.; 7·5–17·5 V/cm; 2, 4 and 6 hr) of that non-dialysable fraction (0·5 mg) in different buffers (0·2 M Tris, pH 7·96; 0·02 M ammonium acetate, pH 8; acetic acid:pyridine:water [5:8:1000; v/v/v], pH 5) showed only a single non-moving spot stained by the Amidoschwarz dye[6] but not by the Oil Red O.[7] The ultra-violet spectrum showed an absorption maximum at 275 mμ (ϵ_{275}: 2·35 for a 1% solution in water). Ultracentrifugation studies (Spinco model E centrifuge, synthetic boundary cell technique) indicated very great homogeneity of the constituents of the non-dialysable fraction. The sedimentation constant ($S^0_{25°C}$) and the mean diffusion coefficient ($\bar{D}_{25°C}$) have been respectively estimated at $1{\cdot}55 \times 10^{-13}$ sec and $5{\cdot}75 \times 10^{-7}$ cm^2/sec^{-1} from two experiments at different concentrations. From those data, assuming a specific partial volume ($\bar{V}$) of 0·76 according to the chemical composition, the molecular weight of the substance(s) has been calculated as 28,000. A high friction coefficient (f/f_0 = about 2·5) and a high viscosimetric increment (about 20 at 25 °C) suggested the substance(s) was extremely hydrated.* Analysis of the non-dialysable fraction gave the following results: nitrogen, 13·4%[8]; lipids, 11%[9]; phosphorus, 1%[10]; hexoses, about 1%.[11] Paper chromatography[11] revealed two hexoses in equal quantities: (a) galactose, (b) glucose or mannose. That constituent would be a phospholipoprotein, the gross chemical composition of which would be in good agreement with the observed physical properties.

One can object that if this phospholipoprotein was really a pure antigen, its activity should be very high. But, of course the substance may have been denaturated in some way during the separation procedure. Present data are to be compared with recent work of Kandutsch[12] about an isoantigenic, triton soluble, lipoproteic fraction.

Acknowledgements—The authors thankfully acknowledge the technical assistance of M. Baron, M. Delhougne, M. Protin, G. Rocoux, N. Thonon, J. Morin and W. Romaniuk. This work was supported by grants from the Fonds National Belge de la Recherche Scientifique, the Fonds de la Recherche Scientifique Médicale Belge, and the Rockefeller Foundation.

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