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On the Production of Immune Sera for Tissues.

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In continuation of studies on cell antigens we examined the production of immune sera for tissues. In the literature there are contradictory reports, but several authors d monstrated marked organ specificity of such sera, *e. g.*, Halpern,[1] Fleisher,[2] and recently Witebsky and Steinfeld.[3] We found that immune sera could be obtained very easily and almost regularly by intravenous administration of small quantities of cell or organ suspensions, *i. e.*, by 3 weekly injections of 10 mg. each of the fresh material suspended in saline. This was done with the hope that in this manner sera of higher specificity could be got than by the injection of larger quantities, as have been used generally. The following materials (ox) were taken as antigens: Tracheal epithelium, thymus cells, kidney and washed sperm cells. The resulting sera differentiated clearly in complement fixation tests between these 4 materials, an example of which is shown in the table.

To 0.5 cc. of progressively doubled dilutions of inactivated serum, starting with 1:10, was added 1 drop of an emulsion of about 2% of the antigen and 0.25 cc. of 1:10 guinea pig serum. After incubation for 1 hour at 37° C., 0.25 cc. of diluted Forssman immune serum containing 2½ hemolytic units and 1 drop of 50% sheep blood were added. The strength of the reactions is indicated as follows: 0 = no hemolysis, tr. = trace, d = distinct, w = weak, str. = strong, v. str. = very strong, ac. = almost complete, c = complete hemolysis.

TABLE.

Antigens	Immune sera for			
	Trachea epithelium	Thymus	Kidney	Spermatozoa
Trachea epithelium	0, 0, 0, 0, str, ac	d, tr, d, ac	str, v. str, ac, c	v. str, ac, c, c
Thymus	0, 0, d, ac	0, 0, 0, d, ac, c	v. str, ac, c, c	str, v. str, ac, c
Kidney	w, str, ac, c	ac, c, c, c	0,0,0,0,0,v.str,ac	d, str, v. str, c
Spermatozoa	ac, c, c, c	c, c, c, c	ac, c, c, c	0,0,0, tr, v.stra,c

On testing, however, with a large number (19) of other tissues group reactions were obtained, more frequently with some sera than

with others, also in cases where there is neither histological nor embryological relationship, *e. g.*, trachea immune serum on brain. Only the sperm immune serum proved to be entirely organ specific. The immune sera were Wassermann negative and had moderate (kidney), little (trachea), or practically no (thymus, sperm) hemolytic activity for ox blood.

[1] Halpern, J., *Z. f. Immunitätsf.*, 1911, xi, 609.

[2] Fleisher, M. S., Hall, T. G., and Arnstein, N., *J. Immunol.*, 1920, v, 437. Fleisher, M. S., and Arnstein, N., *J. Immunol.*, 1921, vi, 223. Fleisher, M. S., *J. Immunol.*, 1922, vii, 51.

[3] Witebsky, E., and Steinfeld, *Centralblatt f. Bakteriologie*, 1927, civ, 144.

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Composition of Bone. II. Analytical Results.

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The micro-technique which we[1] have devised for analyzing small specimens of calcified tissue has been subjected to further test and study. The crushed bones are extracted with ether and alcohol immediately after removal from the body; they are then dried and pulverized. About 10 mg. of bone powder are digested with HCl, trichloracetic acid is added, the solution is made up to 10 cc. and then filtered. Calcium is determined on aliquots of this filtrate. To another aliquot molybdic acid reagent (Briggs-Bell-Doisy) is added, the volume is made up to 10 cc. and then filtered. Inorganic phosphorus is determined on aliquots of this second filtrate. Digestion with HCl of different samples of the same specimen of bone powder gives solutions whose content of calcium and inorganic phosphorus agree as well as duplicate determinations on aliquots of the same solution.

CO_2 is determined on about 20 mg. of bone powder in Van Slyke's manometric gas apparatus.

A specimen of crushed bone was divided into two portions. One portion was extracted with ether and alcohol immediately, the other was allowed to stand unextracted during the summer. After standing for 5 months it was extracted and analyzed. The results for calcium, inorganic phosphorus and CO_2 were the same for both portions. Similar results were obtained with a second specimen of nor-