for all mixed strategies $\mu_1, \ldots, \mu_n$ *and* $i = 1, \ldots, n$ *in an n-person game* Γ *for all possible assignments of the pay-off function h is that* Γ *have total recall.*

Theorem 1 generalizes the theorem of von Neumann which asserts that a zero-sum two-person game with perfect information is strictly determined. It is proved by the same inductive device with a slight variation due to the absence of the minorant and majorant games in the general n-person case. Theorem 2 enables us to replace mixed strategies by behavior strategies in games with total recall and has many computational ramifications. The proofs of both of the theorems and further considerations of extensive games will be published elsewhere.

* Written under a contract with the Office of Naval Research.

[1] Neumann, J. von, and Morgenstern, O., *The Theory of Games and Economic Behavior*, 2nd ed., Princeton University Press, 1947.

[2] A graphical representation by a tree has been suggested by von Neumann, *loc. cit.*, p. 77, however he does not treat this matter systematically, preferring a set theoretical formulation.

[3] In this paper a partition means an exhaustive decomposition into (possibly void) disjoint sets.

[4] It has been noted by von Neumann that Bridge is a two-player game in exactly this manner.

[5] Neumann, J. von, and Morgenstern, O., *loc. cit.*, pp. 67–84.

[6] Neumann, J. von, and Morgenstern, O., *loc. cit.*, pp. 192–194.

[7] Nash, J., and Shapley, L., "A Simple Three-Person Poker Game," *Annals of Mathematics*, Study No. 24 (in preparation).

[8] Kuhn, H., "A Simplified Two-Person Poker," *Ibid.*, Study No. 24 (in preparation).

[9] Neumann, J. von, and Morgenstern, O., *loc. cit.*, p. 51.

[10] Nash, J., "Equilibrium Points in n-Person Games," these PROCEEDINGS, **36**, 48–49 (1950).

THE SPECIFICITY OF ANTI-KIDNEY ANTIBODY DETERMINED BY ITS EFFECT UPON TISSUE CULTURE EXPLANTS

BY RICHARD W. LIPPMAN, GLADYS CAMERON AND DAN H. CAMPBELL

INSTITUTE FOR MEDICAL RESEARCH, CEDARS OF LEBANON HOSPITAL, LOS ANGELES, CALIFORNIA, AND THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY,* CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA†

Communicated by Linus Pauling, August 1, 1950

While investigating the pathogenesis of experimental nephritis produced by rabbit anti-rat-kidney antibody, it occurred to us that the effects of anti-kidney antibody on kidney tissue might readily be visualized in tissue cultures. The specificity of tissue antigens has previously been investigated by the usual immunologic procedures[1] and the effects of antibodies[2, 3] upon tissue explants has long been known. The growth and function of tissue explants have previously been used to study the specificity of tissue

antibodies,[4, 5] but the antisera used were of low, uncertain potency, and only a few different tissues were examined in testing the toxic effects. The following study shows that rabbit anti-rat-kidney gamma globulin is toxic for tissue explants of brain and heart muscle, as well as kidney. These effects are of low species specificity and may be observed in cultures prepared from chick embryo tissues, as well as those prepared from rat tissues. In addition, the serum of patients with glomerular nephritis was found to contain a substance with similar effects and specificity.

Methods.—Rabbit anti-rat-kidney serum was prepared by immunizing rabbits over a period of 4 months with a suspension of rat kidney tissue. The serum was first adsorbed with a suspension of sheep erythrocytes, to remove the Forssman antibodies, and then with rat erythrocytes. The antibody globulin was precipitated by $1/3$ saturation with ammonium sulfate at pH 7.8. This preparation (NTG) was found by the Tiselius pattern to be composed entirely of gamma globulin. The minimal serological activity was determined by a precipitin test with the soluble portion of the original antigen and the preparation NTG was found to contain at least 12% precipitable antibody.

Tissue explants were cultured by the usual double cover slip method, which has been described in detail.[6] In a five-drop portion of culture medium, one drop of NTG (32.5 mg. protein/ml.) was substituted for one drop of serum. Controls were concurrently grown in a medium that substituted one drop of gamma globulin prepared from unimmunized rabbit serum (GC) for one drop of serum. Controls were also grown, in most cases, in a medium of the usual formula without substitution (SC).

Cultures were examined at intervals up to 72 hours, when the experiments were usually terminated, and were graded for growth and evidence of functional activity. At least 4 explants were grown under each condition, and the key conditions were repeated for several groups of 4 explants each. The results were completely uniform. Photographs were taken of typical preparations for permanent record, and some of the tissues were then fixed and stained.

Species Specificity.—Explants of rat kidney in SC and GC grew very well. Those in NTG showed virtually no growth, and in the few cells that grew out from the explant the cytoplasm was granular, with fatty degeneration. Most of these cells were dying, and many became autolyzed during the period of observation.

In order to provide a crucial test of species specificity, explants of chick mesonephros were grown in rat NTG. Almost no growth occurred. In addition, tubular function in the explant, easily visualized by the incorporation of a low concentration of phenol red in the medium,[7] rapidly came to a halt. In contrast, excellent growth and function were observed in SC and GC preparations.

Organ Specificity.—In order to study organ specificity of NTG, tissue explants from different organs of the rat and of the chick embryo were grown in the various media. The results are shown in table 1. As seen from the table, growth of kidney, heart and brain explants was inhibited by NTG. No other tissues were affected, although a wide variety derived from all embryonic layers was examined.

Circulating Nephrotoxin in Patients with Glomerular Nephritis.—It has been well known, since the work of Masugi,[8] that a form of nephritis may

TABLE 1

EFFECT OF RABBIT ANTI-RAT-KIDNEY GLOBULIN ON TISSUE EXPLANTS FROM THE CHICK EMBRYO AND FROM THE RAT

SPECIES	TISSUE	SERUM CONTROL (SC)	GLOBULIN CONTROL (GC)	NEPHROTOXIC GLOBULIN (NTG)
Rat	Kidney	0	0	+
	Heart	0	0	+
	Brain	0	0	+
	Liver	[a]	0	0
	Bladder	[a]	0	0
	Spleen	[a]	0	0
Chick	Mesonephros	0	0	+
	Heart	0	0	+
	Brain	0	0	+
	Liver	[a]	0	0
	Striated muscle	0	0	0
	Lung	0	0	0
	Intestinal smooth muscle	0	0	0
	Intestinal mucosa	0	0	0
	Skin	0	0	0
	Tongue	0	0	0
	Chorio-allantoic membrane	[a]	0	0

+ = marked inhibition of growth, 0 = no effect on growth.

[a] No experiment performed.

be produced in animals by the administration of anti-kidney serum. Lange and his coworkers have reported the occurrence of "auto-antibodies" to kidney tissue in human nephritis.[9] For these reasons, it seemed desirable to test the serum of patients with glomerular nephritis, but without renal failure, for the presence of a circulating nephrotoxin. The serum of 6 patients and 4 normal individuals has been tested in this way, and the results are presented in table 2. Serum from all of the patients with well-documented glomerular nephritis completely inhibited the growth and tubular function of explants from the chick mesonephros. Growth of chick heart

and brain explants was also inhibited. Serum from normal individuals had no effect upon the explants when compared with controls grown in SC.

Comments.—It is of considerable interest that rabbit anti-rat-kidney gamma globulin inhibits the growth in tissue culture of rat heart muscle as well as rat kidney, chick brain, chick heart muscle and chick mesonephros. The presence of a common antigen in these tissues would render reasonable the clinical association of such conditions as glomerular nephritis, rheumatic carditis and chorea.

Even more interesting is the observation that the serum of patients with glomerular nephritis contains a substance which is directly toxic to kidney, heart and brain tissue. Since the patients were selected for adequate renal function, the effect probably cannot be attributed to accumulated waste metabolites in the serum.

TABLE 2

EFFECT ON GROWTH OF CHICK EMBRYO TISSUES OF SERA FROM NORMAL INDIVIDUALS AND FROM PATIENTS WITH GLOMERULAR NEPHRITIS

SUBJECT	DIAGNOSIS	STAGE[10]	MESO-NEPHROS	HEART	BRAIN	STRIATED MUSCLE
R. L.	Normal	...	0	0	0	0
H. U.	Normal	...	0	0	0	0
H. Y.	Normal	...	0	0	0	0
A. M.	Normal	...	0	0	0	0
B. M.	Glom. neph.	Initial	+	+	+	0
A. R.	Glom. neph.	Degenerative	+	+	+	0
K. R.	Glom. neph.	Degenerative	+	+	+	0
D. W.	Glom. neph.	Degenerative	+	+	+	0
C. B.	Glom. neph.	Degenerative	+	+	+	0
W. R.	Glom. neph.	Degenerative	+	+	+	0

+ = marked inhibition of growth, 0 = no effect on growth.

Further experiments to advance all phases of this study are in progress. It is hoped that we shall be able to characterize more completely the organ and species specificity of several tissue antigens and that we shall be able to study in more detail the localization of antibody activity in the various serum protein fractions. Sera from the patients will be fractionated in order to identify the nephrotoxin. It is also hoped that these studies will lead to a simple test for the presence of tissue toxins, and an effort will be made to neutralize such a substance *in vivo*.

The technique of tissue culture offers a sensitive and relatively simple way to examine sera for the presence of tissue toxins and antibodies. It is our ultimate intention to extend these studies to patients with other diseases thought to be related to tissue allergies, such as lupus erythematosus disseminatus, rheumatic fever, rheumatoid arthritis and others.

* Contribution No. 1442.

† This work was aided by a research grant from the National Heart Institute, of the National Institutes of Health. Dr. Lippman is a Fellow of the John Simon Guggenheim Memorial Foundation.

[1] Landsteiner, K., *The Specificity of Serological Reactions*, Harvard Univ. Press, Cambridge, Mass., 1947.

[2] Niven, J. S. F., "The Action of a Cytotoxic Antiserum on Tissue Cultures," *J. Path. and Bact.*, **32**, 527 (1929).

[3] Lambert, R. A., and Hanes, F. M., "The Cultivation of Tissues *in vitro* as a Method for the Study of Cytotoxins," *J. Exper. Med.*, **12**, 453 (1911).

[4] Verne, J., and Oberling, C., "Action des Sérums Cytotoxiques sur les Tissus Cultivés *in vitro*," *Compt. Rend. Soc. Biol.*, **109**, 860 (1932).

[5] Harris, M., "Specificity and Mode of Action of Cytotoxins Produced against Alien Transplants in Rats," *J. Exper. Zoöl.*, **107**, 439 (1948).

[6] Cameron, G., *Tissue Culture Technique*, Academic Press, Inc., New York, 1950.

[7] Chambers, R., and Kempton, R. T., "Indications of Function of the Chick Mesonephros in Tissue Culture with Phenol Red," *J. Cell. and Comp. Physiol.*, **3**, 131 (1933).

[8] Masugi, M., Sato, Y., Murasawa, S., and Tomizuka, Y., "Über die experimentelle Glomerulonephritis durch das spezifische Antinierenserum," *Tr. Jap. Path. Soc.*, **22**, 614 (1932).

[9] Lange, K., Gold, M. M. A., Weiner, D., and Simon, V., "Autoantibodies in Human Glomerulonephritis," *J. Clin. Invest.*, **28**, 50 (1949).

[10] Addis, T., *Glomerular Nephritis*, The Macmillan Co., New York, 1948.

---

*THE EXCITATORY PROCESS IN THE COCHLEA**

BY H. DAVIS, C. FERNÁNDEZ,† AND D. R. MCAULIFFE

CENTRAL INSTITUTE FOR THE DEAF, ST. LOUIS, MISSOURI

Read before the Academy, April 27, 1949, and April 24, 1950‡

Introduction.—The nature of the excitatory process by which sensory cells initiate nerve impulses in afferent nerve fibers is very obscure. In the case of the ear, the most widely accepted theory has been that an electrical potential known as the "cochlear microphonic" is generated by the hair cells in the organ of Corti and serves as a direct electrical stimulus to the peripheral terminations of the fibers of the auditory nerve.

The cochlear microphonic, it will be recalled, seems to be simultaneous with the mechanical movements of the cochlear partition and it follows the wave form of the sound very closely, at least at moderate intensities of stimulation. It shows no refractory period or all-or-none characteristics like the action potentials of nerve.[1] Action potentials of the auditory nerve, probably generated in the cell bodies in the spiral ganglion, not in the fine non-myelinated terminal twigs, can also be recorded from electrodes placed in or near the cochlea.