The effects of aldosterone on adrenalectomized dogs are demonstrated in Fig. 2. Administration of aldosterone restored ability of the distal tubule to lower sodium concentration even in presence of elevated plasma sodium concentration.

Fig. 3 demonstrates the effects of SC-8109 on normal dogs. It is evident that inhibition of distal tubular sodium reabsorption produced by these large doses resembles that produced by bilateral adrenalectomy.

These studies further establish the distal tubule as site of action of aldosterone in sodium reabsorption. They also emphasize the importance of maintaining high plasma sodium whenever assays are made of possible blocking agents.

Summary. In normal dogs the distal tubule can lower Na concentration almost to zero during stop flow, the minimal concentration achieved being independent of plasma Na concentration. In adrenalectomized animals, minimal distal concentrations achieved are not as low. As plasma Na is increased, minimal distal Na concentration also increases in direct proportion, indicating that adrenalectomy reduces maximal Na concentration gradient which can be maintained across distal tubular cells. Results in adrenalectomized and in SC-8109 treated dogs are identical. Administration of cortisone to adrenalectomized dogs does not repair this defect, but aldosterone restores ability of the distal tubule to lower Na concentration, even in presence of elevated plasma Na. Aldosterone when given to adrenalectomized dogs did not alter proximal Na reabsorption, as this can be estimated by stop flow data.

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Activator of Plasminogen and Protease from Various Cell Cultures and Present in Poliomyelitis Vaccine. (25582)

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Previous reports described and characterized an activator of plasminogen produced by KB cells and both an activator of plasminogen and a protease produced by Rhesus monkey kidney (MK) cells(1-4). The present study was undertaken to determine which cell cultures produce these 2 substances and whether they were present in poliomyelitis vaccine prepared from supernatant medium from MK cell culture.

Materials and methods. Cell cultures used are described in Table I. Supernatant medium from cell cultures was obtained as previously described(3). Each supernatant medium was assayed 2 to 5 times for activator and protease using skim milk as substrate(3). Commercially prepared poliomyelitis vaccine, with preservative, without preservative and after dialysis was assayed for activator and protease.

Results. Production of activator and protease by various cell cultures. Cell cultures fell into 3 groups, depending upon production of activator, activator and protease, and detection of no activity in the supernatant medium. As seen in Table I of 21 continuous

TABLE I. Production of Activator and Protease by Various Cell Cultures.*

Produces activator			Produces activator, protease			No activity		
Cell line	Reference	Growth medium	Cell line	Reference	Growth medium	Cell line	Reference	Growth medium
KB†	Eagle	199-10% CS	MK†	(primary)	199-2% CS	LAC†	Baron, Rabson	199-10% HS
KB‡	"	BME 10% HS	RK†	"	"	MS†	Tytell, Melnick	199-10% CS
HeLa†	Gey	199-10% CS	HK†	"	199-10% CS	HH‡	Girardi	BME 10% HS
HeLa S-3‡	" , Puck	BME 10% HS				Detroit-98‡	Berman, Stulberg	"
HeLa‡	Gey	"				Detroit 6‡	*Idem*	"
HC‡	Chang	BME 20% HS				D-189‡	Leighton	"
Monkey heart†	Salk	199-10% CS				Human embryonic intestines‡	Henle	"
RK†	Drew	199-20% CS				Liver‡	Chang	"
MAF‡	Gray	BME 10% HS				Sarcoma 180‡	Foley	BME 10% ES
Monkey thyroid primary†	Hare	BME 20% HS				J-111‡	Osgood	BME 10% HS
						Bovine kidney‡	Mai	BME 10% CS
						Bovine skin & muscle‡	"	"

* Abbreviations are as follows: CS = calf serum, HS = human serum, HC = human conjunctiva, BME = basal medium, Eagle, MK = monkey kidney, RK = rabbit kidney, HK = human kidney, ES = horse serum, HH = human heart, Mai = Microbiological Associates, Inc.
† Grown at DBS, NIH, by Dr. J. G. Crawford and George Gardner.
‡ Obtained from Microbiological Associates, Bethesda, Md.

cell lines examined, 9 produced only activator (2 to 256 units/0.2 ml), none produced protease, and 12 had no activity as activator or protease. Different lots of 3 strains of HeLa cells produced varying amounts of activator and some lots produced none. Of the 4 primary cell lines tested, 3 of kidney origin produced both activator (32 to 4096 units/0.2 ml) and protease (2 to 256 units/0.2 ml). Production of protease by kidney cell culture was reported earlier(2,4).

Activator of plasminogen and protease in poliomyelitis vaccine. Eighty-three lots of poliomyelitis vaccine were tested for activator and protease. Activator alone was present in 29 lots, activator and protease was present in 8 lots while 46 lots had no activity. The amounts of activator and protease in vaccine were generally lower than the amounts in MK supernatant medium. Results were similar whether the lots of vaccine tested contained preservative, were without preservative or were dialyzed.

To determine if presence of these proteolytic materials in vaccine affected antigenic potency, monkey potency ratios(5) of all lots tested were reviewed. There was no relationship between presence of proteolytic materials and antigenicity for monkeys.

Discussion. The data indicate that activator for plasminogen and protease were produced by many continuous and primary cell cultures. Simultaneous production of activator and protease was limited to 3 primary cell cultures which originated from kidneys of 3 different species. Production of activator and protease and possibly other biologically active materials by cell cultures may be of value for identification and classification of these cultures.

The presence of active biological substances in poliomyelitis vaccine indicates that these substances resist destruction by inactivation process to which the vaccine is subjected. Similar substances, as tryptic leucoprotease, have been demonstrated to be highly resistant to formalin(6). Heat stability studies show that temperatures to which vaccine is subjected for inactivation would not destroy these proteolytic substances. These data show that active materials other than virus antigen occur in poliomyelitis vaccine. The high levels

of protease inhibitors in sera and body tissues (7) make the presence of proteolytic substances in vaccine of doubtful clinical significance. Similarity of activator from tissue slices and activator produced by cell cultures has been noted(3). It has been proposed that fibrinolytic conditions *in vivo* are due to the activator from tissue slices(8). Most of the activator produced by cell cultures is released into the supernatant medium. It is possible that cells *in vivo* may release activator into extracellular fluid and be responsible for fibrinolytic conditions *in vivo*.

Summary. Assays of 25 metabolizing cell culture lines disclosed that 13 of these released an activator of plasminogen resembling tissue activator. Of 4 primary cell lines tested the 3 of kidney origin produced both activator and protease. Assay of a specific cell culture fluid, commercially prepared poliomyelitis vaccine, disclosed the presence of both activator and protease in many lots of vaccine.

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Enzymatic Determination of Glucose-6-phosphate in Tissue Extracts Containing Glutathione and Other Interfering Substances.* (25583)

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Enzymatic reduction of triphosphopyridine nucleotide (TPN) with glucose-6-phosphate dehydrogenase (G-6-PDH) can be used for quantitative determination of glucose-6-phosphate (G-6-P)(1). However, most preparations of G-6-PDH are contaminated with other enzymes which interfere with accurate determination of G-6-P, especially when carried out in crude tissue extracts. In the present report are described conditions under which G-6-P may be measured in tissue extracts by use of sufficiently purified G-6-PDH preparations and the selective inhibition of both hexokinase and glutathione reductase.

Methods. Three preparations of yeast G-6-PDH were gifts from C. F. Boehringer and Sons (Mannheim). A pure crystalline barium salt of G-6-P from Sigma Chemical Co. was converted to potassium salt before use. Oxidized glutathione (GSSG) was prepared from reagent grade glutathione (GSH) of Fisher Scientific Co. by the method of Rall and Lehninger(2). Formation of reduced TPN (TPNH) was followed at 340 mμ in Beckman DU Spectrophotometer, using 1 ml cell with light path of 1 cm. The reaction was carried out at room temperature in *tris*-(hydroxymethyl)aminomethane (Tris) buffer at pH 8.3 with sufficient G-6-PDH to oxidize 0.03 μmole of G-6-P in about 1 to 3 minutes in the presence of inhibitors to be described below. The blank solution contained Tris and the inhibitors but no enzyme. The reaction was initiated by addition of G-6-P or tissue extract to experimental and blank solutions, which brought the volumes to 1 ml. The initial baseline reading, corrected for dilution by substrate, was subtracted from the stable final reading, and the change in optical density was divided by 6.22 to obtain μmoles of TPNH

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