gen-antibody reaction is, therefore, suggested and warrants further study.

Summary. A phospholipase A preparation from cobra venom *(Naja naja)*, made by heating the venom in neutral or acid solution at 100°C for 15 minutes, released histamine and SRS activities from chopped guinea pig lung, as did the unheated venom. The direct lytic factor of the venom which is required for venom hemolysis did not appear to be necessary for this release. Lysolecithin, which is formed by the action of phospholipase A on lecithin, also released histamine and SRS activities from chopped guinea pig lung. Suspensions of homogenized or lyophilized guinea pig lung did not release SRS activity when kept at 0°C but did release this activity when incubated at 37°C.

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Quantitative Microdetermination of Enzymes in Sweat Gland III. Succinic Dehydrogenase in Cystic Fibrosis.* (30213)

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Various metabolic enzymes in sweat glands are being assayed in an attempt to discover the basic defect in cystic fibrosis. Manifestations of this disease include an excessive concentration of sodium chloride in sweat, representing failure to achieve the normal hypotonicity with respect to extracellular fluid. The abnormality is not understood, but may involve failure of a "sodium pump" mechanism concerned with retrieval of sodium and chloride.

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Alkaline phosphatase, acid phosphatase, adenosine triphosphatase, cholinesterases, and isocitric, glucose-6-phosphate, lactic, and malic dehydrogenases have been explored in the present series(1,2). This communication concerns succinic dehydrogenase (SDH). This enzyme is of special interest due to its association with various absorptive and secretory organs. It represents an analytical difficulty, in that it is easily destroyed in the process of freezing-drying. Chamness *et al* reported that "enzymes in hydrogen transport are damaged by drying" as indicated by failure of frozen-dried skin to demon-

strate reduction of tetrazolium (3,5, triphenyl tetrazolium chloride)(3). Padykula reported that histological evidence of SDH activity, in tissue incubated anaerobically, was better observed in moist sections than in dried, but that activity could be partially restored in the latter with Ca and Al ions, cytochrome C or denatured globin(4). In spite of the lability of SDH in the freezing-drying process, the present study concerns some activity found in sweat gland tissue isolated by microdissection following a freezing-drying procedure.

Method of enzyme assay. Skin biopsies were taken by means of a high speed rotary drill. These were imbedded in tragacanth jelly and frozen at —20°C. They were sectioned at 24 μ thickness on the cryostat microtome. Each slice was placed in a separate compartment of an aluminum holder and desiccated *in vacuo* at cryostat temperature for one hour. The reduced pressure reached 23 $\pm$ 6 μ. The tissues were brought to room temperature while under vacuum. Pieces of sweat gland were separated by microdissection and weighed on a quartz fiber "fishpole" balance.

SDH activity was assayed by an adaptation of the method of Defendi and Pearson (5). The weighed pieces of sweat gland, ranging from 1.2 to 10.8 μg, were placed in small test tubes. The tubes were placed in an ice bath and 0.1 ml of cold (4°C) buffer-substrate mixture was added. The buffer-substrate was a freshly prepared mixture and contained one part 1.5 mg p-iodonitrotetrazolium violet per 1 ml water (Defendi and Pearson used 1 mg per ml, but we obtained a more sensitive reaction with use of 1.5 mg per ml); one part 0.1 molar phosphate buffer, pH 7.4; and one part 0.1 molar sodium succinate. The digestion mixture was incubated in a Dubnoff shaking incubator for 1 hour at 37°C, after which the tubes were immediately returned to the ice bath and 0.5 ml cold ethyl acetate were added. The mixture was allowed to stand at room temperature for 10 minutes with 3 thorough mixings with a Vortex mixer during this period to achieve complete extraction. The solutions were then centrifuged for 5 minutes at 2,000 r.p.m. and read in Pyrocell microcuvettes in the Beckman DU spectrophotometer at 494 mμ.

For preparation of a standard curve, tetrazolium was reduced by an excess of ascorbic acid and the colored formazan was extracted with ethyl acetate.

Special characteristics of SDH activity. When fat in the tissue slices came into contact with the sweat gland tissue, SDH activity seemed to be decreased. This is in accordance with the findings of Hershey who states that "the use of mineral oil, vaseline or other lubricants frequently used in cutting human grafts must be avoided."

For an indication of the amount of loss of activity due to freezing and drying, a study was made using mouse liver as the enzyme source. An aqueous homogenate was prepared containing 20 μg of liver per 100 μl of homogenate. As shown in Table I, fresh liver contained the amount of SDH activity to split 0.154 mole of substrate per kg wet liver per one hour incubation. This is read as colored formazan. With each mole succinate split, 1 mole tetrazolium is reduced, thus yielding 1 mole of formazan. Exposing similar samples of homogenate to the freezing-drying process, it yielded 0.038 mole formazan. Weighed pieces of unhomogenized freeze-dried liver gave an activity of 0.096 mole. Samples of fresh mouse liver were weighed wet and again after freezing and vacuum-drying one hour with an average weight decrease of 67%. (Twelve samples were done with weight decrease ranging from 60% to 78%.) Use of this figure to convert from activity per dry weight to activity per wet weight in the last 2 columns of Table I gave a result of 0.032 mole activity per wet weight. It is thus concluded that use of unhomogenized fragments of liver tissue in the freezing-drying process, as done for the sweat gland tissue, results in a residual activity of approximately 20.5% of the original activity. How closely this determination on liver tissue would apply to sweat gland tissue is not known.

The question arose as to whether this 20% residual was actually SDH activity. Liver homogenate was, therefore, exposed to

TABLE I. Succinic Dehydrogenase in Mouse Liver, with Effect of Freezing and Drying.

All determinations done in triplicate and reported as moles of substrate split per kg tissue per hour incubation.

Mouse No.	A Fresh homog (M/kg wet wt/hr)	B Frozen-vac homog (M/kg wet wt/hr)	C Frozen-vac liver plug (M/kg dry wt/hr)	D Frozen-vac liver plug calculated as wet wt (M/kg wet wt/hr)
1	.121	.021	.064	.021
2	.130	.037	.098	.032
3	.209	.063	.156	.051
4	.157	.031	.067	.022
Mean	.154	.038	.096	.031

Column A—Freshly prepared homogenate without freezing or drying.

Column B—Frozen and vacuum-dried homogenate.

Column C—Liver without homogenizing, frozen-dried and weighed. At the same time as the homogenate was prepared, pieces of liver tissue were imbedded in tragacanth, frozen, cut, vacuum dried and pieces were dissected and weighed. The liver was prepared the same as sweat gland tissue.

Column D—Experimentally, we have found that the actual weight of liver decreases approximately 67% due to vacuum freezing-drying. Column D is the calculated figure of the dried liver sections as if determined as wet weight—actually it is 33% of Column C. This figure very nearly approximates the result in Column B, indicating that there is no great difference in activity if the determination is done on frozen-dried liver homogenate or on frozen-dried liver tissue fragments.

room temperature over varying periods. The activity was found to decrease with time and to have completely disappeared after 48 hours at room temperature. Other samples were exposed to boiling water temperature and this decreased the activity to approximately 1/100 of the original. These observations indicate that the 20% residual activity is due to a labile factor, presumably succinic dehydrogenase.

Results and discussion. Succinic dehydrogenase activity was determined in sweat gland tissue of 7 cystic fibrosis children and 7 control children. As indicated in Table II, the activity in cystic fibrosis patients was not significantly different from that in the control children. No quantitative data on SDH activity in isolated sweat gland have been found in the literature. Determinations on whole skin gave results similar to those reported by Hershey(6).

Data on the SDH content of mouse liver have been reported from other laboratories. Nachlas *et al* reported activity of 79 and 105 units per mg liver (wet weight) in 2 mice. They defined one unit as the amount of enzyme which will yield 1 μg of formazan per 15 minutes incubation per mg of liver. By converting our mean value (0.154 mole substrate split per kg liver weight per hour)

TABLE II. SDH Activity in Sweat Glands of Cystic Fibrosis Patients and Controls.

Moles of substrate split per kg of dry sweat gland per hour incubation. Three determinations were done on each patient.

Cystic fibrosis		Control	
K.B.	.030	F.B.	.022
	.056		.037
	.034		.027
B.B.	.027	C.W.	.023
	.045		.068
	.045		.035
R.C.	.054	R.C.	.045
	.050		.026
	.038		.023
R.S.	.023	J.H.	.023
	.028		.036
	.038		.045
G.S.	.056	R.I.	.045
	.045		.056
	.064		.034
L.G.	.063	R.R.	.054
	.045		.063
	.063		.060
R.T.	.054	J.U.	.068
	.045		.068
	.051		.045
Mean	.045 ± .012		.043 ± .016

Cystic fibrosis patients were aged in years as follows: K.B. 9, B.B. 3, R.C. 12, R.S. 7, G.S. 15, L.G. 10, R.T. 5.

The ages in years and diagnosis of the controls were as follows: F.B. 8, mumps encephalitis, C.W. 13, normal, R.C. 4, hydrocephalus, bronchitis, J.H. 12, normal, R.I. 4, urinary tract infection, R.R. 10, glomerulonephritis, J.U. 6, glomerulonephritis.

to those of Nachlas *et al*(7), we obtain a value of 17.8 units. Barka and Dallner reported a mean of 7 mg formazan reduced per g wet weight in 30 minutes(8). Our values for 30 minutes would be approximately 35 mg. Kun *et al* reported 1.6 μg dye reduced per mg liver in 10 minutes(9). For this unit, our value would be approximately 12.4 μg. Our values are thus within the wide range as reported by others.

Summary. A micro method for quantitative determination of succinic dehydrogenase (SDH) activity was applied to frozen, vacuum-dried sweat gland. SDH activity determinations were made on cystic fibrosis children and controls. No significant difference between the two groups appeared.

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Stimulation of Gastric Acid Secretion by Acetate. (30214)

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Although ethyl alcohol has long been known to stimulate gastric acid secretion, there is no agreement concerning the mode of action. Dragstedt *et al*(2) observed that perfusion of organs with dilute alcohol solution resulted in histamine-like activity in the perfusate. They considered histamine release to be a possible mechanism. However, Irvine, Watkin and Williams(6) did not detect a rise in free histamine concentration in the urine during alcohol stimulation but a histamine infusion producing equivalent acid response raised urinary histamine levels. Woodward, Slotten and Tillmanns(12) reported that alcohol applied directly to antral mucosa was a powerful stimulant for the antral gastrin mechanism. Irvine, Watkin and Williams(6), although confirming the gastrin releasing effect of ethanol, did not believe that gastrin was the major factor mediating the acid response to oral or intravenous alcohol. Hirschowitz *et al*(5) interpreted results of experiments with human subjects to indicate a central mechanism for alcohol stimulation of gastric secretion mediated through the vagus nerve.

The stimulatory effect of acetate, a metabolic product of ethanol, was reported by McManus(11). Gastric stimulation by acetate was used by Zawoiski, Braunschweig and Beyer(13) in experiments with steroids. Certain similarities between acetate and ethanol stimulation were observed. In this paper are presented in more detail some of these observations.

Methods. Six female Heidenhain gastric pouch dogs with total antrum resections were used in the experiments. The dogs ranged in weight from 10.0-12.7 kg. The animals were prepared surgically several months before they were used experimentally. Test meals determined the adequacy of resection of antrum. The animals had been trained to stand in a loose sling and were not anesthetized during experiments. Collections of gastric secretion were made into 12 ml graduated centrifuge tubes attached to stainless steel gastric cannulas. Acidity of collections was measured with a Beckman glass electrode pH meter. Since the small volumes of some of the collections limited the analysis, gastric acidity was expressed in terms of pH. The