been tested with the majority falling into Types 2 and 4. None so far has corresponded to Type 3 of Dalldorf.(2) A later report will be made when the tests for all of the strains on hand have been completed.(4) The results with the human sera were not generally so clear cut. Twenty-two pairs of acute and convalescent sera were tested against antigens of Types 1 and 2. Typical results for 8 pairs are given in Table II. An occasional rise in titer for the same strain was noted in both tests. Often the antibodies were present soon after the onset. In general, there was agreement between the two methods as to the antibody types found. If the neutralization test was positive for Type 1, for instance, the complement fixation test was positive for the same type. Occasionally antibodies were found for both types in each test.

Summary. The complement fixation test using filtered muscle antigen can be employed for differentiation of the Coxsackie virus into serologically distinct types and for determining the presence of antibodies in human sera.†

4. Hewitt, B. F., in manuscript.

† Since submitting this paper for publication, Casals, Olitsky and Murphy also have reported on the use of the complement fixation test for differentiating the Coxsackie viruses. PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 636.

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A Short Column Procedure for Separating Cytoplasmic Components from Normal and Tumor Tissue. (17587)

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Columnar chromatography has been shown to be applicable to purification and characterization studies of certain subcellular particulates of wide size and physiological variation, namely the virus-like agent of chicken tumor I(1) and the microscopically visible granules of the Harding-Passey and the S91 mouse melanomas.(2) Most of these studies were made with material that had already been partially purified by differential centrifugation. It has since been found, in working with the latter tissues, that the preliminary purification can be done rapidly without recourse to the centrifuge by employing a procedure involving grinding of the tissue with diatomaceous silica (Celite No. 503) and submitting this slurry to a short column or pad of the same adsorbent. The resulting percolate consists essentially of the subcellular granules and most of the soluble components of the tissue extract. It is devoid of nuclei and intact cells as indicated by microscopic examination. This tissue percolate is suitable for certain enzyme investigations or further purification studies by chromatography or differential centrifugation.

Methods and results. A typical procedure is outlined in the following experiment indicating the relative results obtained with equal quantities of tumor tissue prepared by the short column technic as compared with standard grinding followed by centrifugation. Several Harding-Passey tumors were pooled and fragmented by forcing through a 5 ml syringe without needle, and divided into 2 equal lots of 5 g each termed A and B. Lot A was ground in an iced mortar without sand and with the gradual addition of 5 times its weight of cold distilled water. This suspension was then cleared of tissue debris, unbroken cells, and nuclei by two successive centrifugations† of 5 minutes each at approxi-

* With the aid of John Chambers.

1. Riley, Vernon T., *Science*, 1948, v107, 573.

2. Riley, Vernon T., Hesselbach, M. L., Fiala, S., Woods, M. W., and Burk, D., *Science*, 1949, v109, 361.

† Type SS-1 Serval centrifuge with 9″ angle rotor, run at approximately 3700 rpm in a walk-in refrigerator at 5°C.

TABLE I.
Comparison of Harding-Passey Fractions Prepared by Centrifugation and the Short Column Procedure.

Criteria	Extract		Resuspended pellets	
	(A) Centrifuge	(B) Short column	(A) Centrifuge	(B) Short column
Grams of tumor	5.0	5.0	—	—
Vol. of extract, ml	50.0	50.0	—	—
Optical density (× 10) 650 mu	10.6	13.0	9.2	11.5
Dry wt, mg/ml	10.4	11.6	4.2	4.5
Nitrogen, mg/ml*	1.01	1.14	0.41	0.42
Phosphorus, mg/ml*	0.10	0.12	0.03	0.03
Succinoxidase activity†				
μl O_2/hr	237.5	248.6	159.0	167.9
QO_2	22.8	21.4	37.9	37.3
$QO_2(N)$	235.1	218.1	387.8	399.8
Dopa oxidase activity‡				
μl O_2/hr	287.6	332.0	100.0	102.4
QO_2	27.7	28.6	23.8	22.8
$QO_2(N)$	284.8	291.2	243.9	243.8

* Determinations made in the NIH micro-chemical laboratory under the supervision of Mr. William C. Alford.

† Succinoxidase activity was determined manometrically with the following vessel contents: 1.0 ml of tissue fraction, 0.2 ml of succinate-cytochrome C mix composed of 1/3 commercial cytochrome C (VioBin) and 2/3 sodium succinate, 0.5 M; 0.1 ml 2N KOH in center well. All experiments were run at 38°C. The values were calculated from the first 30 minutes uptake.

‡ Dopa oxidase activity was determined as above except 1 mg of dopa in 0.2 ml distilled water was substituted for succinate-cytochrome C in the sidearm.

TABLE II.
Comparison of S91AA Amelanotic Tumor Fractions Prepared by Centrifugation and by the Short Column Procedure.

Criteria	Extract		Resuspended pellets	
	(A) Centrifuge	(B) Short column	(A) Centrifuge	(B) Short column
Grams of tumor	5.0	5.0	—	—
Vol. of extract, ml	50.0	50.0	—	—
Nitrogen, ml	0.91	1.18	0.18	0.23
Succinoxidase activity				
μl O_2/hr	148	167	69	88
$QO_2(N)$	162	141	372	374
Cytochrome oxidase activity*				
μl O_2/hr	240	330	266	277
$QO_2(N)$	262	280	1440	1180

* Determined in the same way as succinoxidase activity except that PPDA was substituted for succinate at a vessel concentration of 0.02 molar.

mately 1500 g.(3) The supernatant fluid was diluted with cold distilled water to 50 ml and called extract A.

Lot B was ground in a mortar in the same manner as batch A with the exception of 5 g of No. 503 Celite was gradually added to the tissue slurry as the grinding proceeded. This thick suspension was poured on a short broad column of silica prepared by adding 2 g of Celite in suspension to a 6 cm Buchner funnel containing a No. 31 Whatman filter paper. The excess water was withdrawn with negative pressure, care being taken not to dry the Celite to the point of shrinkage. After the thick

3. Claude, A., *J. Exp. Med.*, 1946, v84, 51.

TABLE III.
Comparison of Normal Mouse Liver Fractions Prepared by Centrifugation and by Short Column Procedure Employing 30% Sucrose.

	Extract		Resuspended pellets	
Criteria	(A) Centrifuge	(B) Short column	(A) Centrifuge	(B) Short column
Grams of liver	6	6	—	—
Vol. of extract, ml	60.0	60.0	—	—
Nitrogen, mg/ml	1.76	1.90	0.51	0.44
Succinoxidase activity				
μl O_2/hr	207	221	178	147
$QO_2(N)$	118	116	347	334
Cytochrome oxidase activity				
μl O_2/hr	272	274	187	171
$QO_2(N)$	155	144	365	388

slurry of diatomaceous silica and ground tissue was added to the prepared funnel, negative pressure was cautiously applied. Just before the column went dry an additional 25 ml of cold distilled water was added in increments to further elute the granules from the silica. The resulting percolate from lot B was diluted to 50 ml and tested against extract A. In addition to a comparison of the extracts obtained by the two methods, an aliquot of each was sedimented at high speed in the centrifuge (20,000 g for 15 minutes) so that the particulates obtained could be tested free of soluble components in the supernatant fluid. The resulting pellets were resuspended in cold distilled water to the starting volume and tested along with the extracts. By phase contrast microscopy the pellet fractions appeared to consist essentially of granules 0.2 to 1.0 μ in diameter, though some submicroscopic particulates undoubtedly were present. All fractions were tested in respect to recovery in terms of dry weight, nitrogen, phosphorus, and optical density at 650 mu; and for biological activity in terms of the dopa and succinoxidase enzyme systems. The results are given in Table I, and demonstrate that the two methods agree well in regard to dopa oxidase and succinoxidase activity and the other criteria listed.

Though this method has been employed primarily in the preparation of melanoma extracts, other tumors and mouse liver mitochondrial preparations have been explored. Preliminary results suggest that the technic may have general application with appropriate minor modifications. Tables II and III show the results obtained when employing the technic to prepare mitochondrial containing extracts from an amelanotic tumor‡ and normal mouse liver. The tumor fractions were prepared by the procedure outlined above, whereas the liver procedure was modified by employing 30% sucrose,(4) in lieu of distilled water, and by reducing the Celite used in grinding to 2.5 g. The liver extracts obtained by the two technics were microscopically similar. Both preparations contained filaments, short rods, and spherical granules, though semi-quantitative estimates with a counting chamber indicated a slightly greater population of long filaments in the centrifuge preparation. Examination of both extracts with the phase contrast microscope failed to reveal visible particulates other than those resembling mitochondrial elements. The column extract was somewhat pinker, probably due to the fact that more red blood cells were lysed by the Celite grinding. These pigments were largely eliminated, however, by subsequent centrifugation, and it has previously been shown that blood pigments can be largely, if not entirely, removed by column chromatography.(2)

Summary. The short column technic described is an adjunct to the chromatographic methods previously published(2) and makes

‡ S91-AA tumor derived from the Cloudman S91 mouse melanoma by Algire.

4. Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., *J. Biol. Chem.*, 1948, v172, 619.

available a complete procedure for the preparation of certain subcellular particulates from the crude tissue to the purified fractions without recourse to the centrifuge at any stage.

Some of the advantages of the short column technic for the preliminary preparation of cytoplasmic fractions are speed, simplicity of equipment and operation, an adaptability to a controlled gas phase during manipulation, and a sharp separation of cytoplasmic elements from nuclei and viable cells.

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On the Insulin Content of Pancreatic Juice.* (17588)

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It has been suggested that the islets of Langerhans are connected with and regenerated from duct tubules.(1) It is of interest to determine whether or not the internal secretions of the islands are present in the external pancreatic secretion. We have attempted to demonstrate insulin in fresh pancreatic juice by means of the "insulin effect" on rat diaphragm *in vitro*. The uptake of glucose by the isolated rat diaphragm has been extensively studied by Gemmill,(2) Stadie and Zapp,(3) Perlmutter and Greep,(4) Villee and Hastings, (5) and others. They have shown that glycogen synthesis by the rat diaphragm (measured directly or by glucose disappearance from the medium) is increased by the addition of insulin to the substrate.

Methods. Extraction. Pancreatic juice was extracted by modification of the insulin extraction procedures of Somogyi *et al.*(6) and Best and Scott.(7) Juice from the cannulated major pancreatic duct of the dog was collected into acid alcohol (95% ethyl alcohol plus 1.3% acetic acid) in proportion two parts of juice to three parts of acid alcohol. When 400 to 600 ml had been collected (usually over two to three days) the mixture was brought to pH 7.5-8.0 with several milliliters of 1 N NaOH and filtered. The filtrate was adjusted to pH 8.0 and concentrated *in vacuo* at 25-28°C for 24 to 48 hours. The residue was taken up in 50 to 100 cc of water, brought to pH 8.0 with .01 N NaOH, and filtered. To the filtrate was added ammonium sulphate 40 g/100 ml and the precipitate allowed to settle out overnight in the ice box. The precipitate was collected in a Buechner funnel on hard filter paper and then eluted repeatedly with 80% ethyl alcohol. The addition of an equal volume of ethyl ether caused a precipitate to appear. By repeated isoelectric precipitation was isolated that fraction soluble in water at pH 3.5 and 8, and insoluble at pH 5.0. This fraction was taken up in small amounts of water, buffered, and used for assay. To certain portions of pancreatic juice commercial insulin was added and the extraction carried out as above.

Assay. Well fed adult rats were killed by hammer and diaphragms quickly excised and placed in cold saline. Quarter diaphragms weighing 50 to 100 mg were placed in Warburg flasks. Each flask contained 2.0 ml of Krebs-Ringer phosphate solution buffered to pH 7.4, and containing 100 mg% of glucose, and 0.02

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5. Villee, C. A., and Hastings, A. B., *J. Biol. Chem.*, 1949, v179, 673.

6. Somogyi, M., Dorsy, E. A., and Shaffer, P., *J. Biol. Chem.*, 1924, v60, 31.

7. Best, C. H., and Scott, D. A., *J. Biol. Chem.*, 1923, v57, 709.