of pepsitensin and hypertensin by means of paper electrophoresis (*6*), using a slight modification of Durrum's technique (*7*). Three strips of No. 1 Whatman filter paper (2.5 by 40 cm) were suspended by their central parts on a horizontal supporting glass rod and their ends were dipped into positive and negative electrode vessels containing a glycine/HCl buffer solution of *p*H 2.5 and 0.1 ionic strength.

After the strips were soaked with the buffer solution, a potential difference of 3500 (*8*) was established between the two poles and the current was maintained for 30 to 60 min and then interrupted. Ten microliters of equivalent solutions (130 Indianapolis units/ml) of pepsitensin and hypertensin was then placed on the center position of strips 1 and 2, respectively, and a mixture containing 5 µlit of each solution was applied to strip 3. The whole system was placed in a chamber where air was completely replaced by nitrogen. The electric current was maintained for 5 to 6 hr, the voltage being kept constant through a voltage regulator; the current usually rose from 6 ma at the beginning of the experiment to 8 ma at the end. The paper strips were then dried at room temperature and cut into pieces 1 cm long that were assayed on the isolated guinea-pig ileum.

The response of this preparation has been shown to be a very sensitive method for the detection of hypertensin (*9*) and pepsitensin (*10*). As Fig. 1 shows, both substances migrated toward the cathode at *p*H 2.5; but pepsitensin had a greater mobility than hypertensin, and they were separated when a mixture of the two was submitted to electrophoresis. Thus, hypertensin and pepsitensin seem to be such similar peptides that their mixture cannot be resolved by paper chromatography with three pairs of solvents, but it can be resolved through paper electrophoresis.

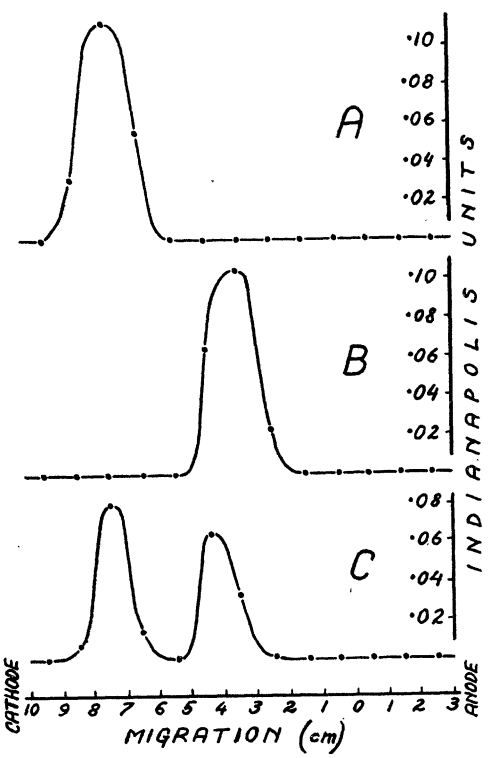

Fig. 1. Migration of pepsitensin, hypertensin, and a mixture of both on paper electrophoresis: *A*, pepsitensin; *B*, hypertensin; *C*, pepsitensin plus hypertensin. Potential difference, 350 v; time, 6 hr; buffer glycine/HCl, *p*H 2.5, ionic strength 0.1. The substances were localized by testing segments of paper on the isolated guinea-pig ileum.

References and Notes

1. H. Croxatto and R. Croxatto, *Science* **95**, 101 (1942).
2. E. Braun-Menendez *et al.*, *Renal Hypertension* (Thomas, Springfield, Ill., 1946).
3. E. Braun-Menendez *et al.*, *Rev. soc. argentina biol.* **19**, 304 (1943).
4. M. Rioseco Vasquez, "Nueva contribución al estúdio de la pepsitensina," thesis, Universidad Católica, Santiago de Chile, 1945.
5. A. C. M. Paiva, T. Bandiera, and E. S. Prado, *Ciencia e cultura* **5**, 273 (1953).
6. Work subsidized by the Conselho Nacional de Pesquisas, Brazil.
7. E. L. Durrum, *J. Am. Chem. Soc.* **72**, 2943 (1950).
8. We are grateful to M. D. Souza Santos, Department of Physics, University of São Paulo, for the electric part of our electrophoresis apparatus.
9. Z. P. Picarelli *et al.*, *Circulation Research*, in press.
10. A. C. M. Paiva, thesis, Escola Paulista de Medicina, in press.

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A Method of Assessing Experimental Pulmonary Edema

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A technique for assessing pulmonary edema has been successfully used at Medical Laboratories in recent months. The original technique was first shown to me some years ago by Hermann Rahn (*1*) of the University of Rochester, whose interest in respiratory physiology has led him, among numerous other activities, to preserve the lungs of a great number of species of animals by drying them on a compressed-air line. Lung, when minus tissue water and blood and inflated with air, becomes parchment-like yet preserves its morphological features and remains intact for many months with no other treatment. After some years of using this technique to make preparations, it has been adapted to the study of the dynamics of edema formation.

In order to measure the rate of development and the amount of pulmonary edema produced by chemical or physical agents in experimental animals, one must know the weight of the nonedematous lungs. This value is usually calculated from average figures taken from a large sample of untreated animals. Since the weight of the lung as percentage of body weight is quite variable among individuals of all species, obtaining an average figure means the use of considerable numbers of control and experimental animals in order to obtain a reliable figure. We are describing

Table 1. Relationship of lung weights to time after exposure.

Treated					Untreated			
Body wt. (g)	Lung wt. (g)		Difference (g)	Exposure time (hr)	Body wt. (g)	Lung wt. (g)		Difference (g)
	Wet	Dry				Wet	Dry	
183	1.7	0.32	1.38	1	510	4.3	0.9	3.4
345	3.6	.59	3.01	1	280	2.3	.5	1.8
335	3.7	.605	3.09	1	285	2.7	.5	2.2
670	4.4	.95	3.45	1	730	6.0	1.4	4.6
720	6.8	1.5	5.3	1	332	2.2	0.4	1.8
580	5.15	1.15	4.0	1	550	5.0	1.1	3.9
			3.37	Mean	680	5.4	1.2	4.2
220	3.0	0.44	2.56	2	760	5.7	1.1	4.6
317	5.3	.70	4.6	2	660	7.0	1.4	5.6
279	5.4	.635	4.76	2	440	3.7	0.7	3.0
309	3.6	.535	3.06	2	550	5.1	1.1	4.0
400	5.2	.71	4.49	2	700	4.8	1.1	3.7
387	4.6	1.06	3.54	2	420	2.9	0.6	2.3
670	8.0	1.11	6.89	2	480	3.3	.7	2.6
			4.27	Mean	382	2.7	.6	2.1
450	4.3	0.93	3.37	4	290	2.6	.45	2.2
375	8.4	.87	7.53	4			Mean	3.25
468	6.4	1.56	4.84	4				
620	13.0	1.3	11.7	4				
510	8.3	1.155	7.14	4				
630	12.8	1.66	11.14	4				
			7.62	Mean				

here a method whereby each animal provides its own control value, thus enabling one to consider events in each individual and to reduce greatly the number of animals necessary per experiment.

The physical manipulations involved are extremely simple. The thoracic organs (including trachea as far craniad as the thyroid cartilage) are removed, and the lungs are separated from heart, great vessels, and lymph nodes. Care is taken not to rupture any portion of the bronchial tree. The lungs are then blotted on toweling or filter paper to remove any adhering blood; no attempt is made to drain completely the smaller pulmonary and bronchial vessels at this time. The weight is obtained. A cannula is inserted into the trachea and securely tied. The cannula is then attached to a source of compressed air (preferably in series with a cotton or glass wool filter) and inflated to a firm consistency. This degree of inflation often brings the lungs to somewhat greater than life size. As blood and edema fluid are forced out of the tissue and vessels by expansion of the organ, they are gently removed with gauze sponges, and the normal preparation then has a uniform pink color. The lungs are left inflated for 12 to 24 hr, depending on the gross size, at which time they are firm, smooth, and dry. When completely dried, the cannula is removed and the lungs are weighed again.

A series of guinea pigs treated percutaneously with 20 mg/kg of an aqueous 70-percent solution of phosgene oxime (*2*) and sacrificed at time intervals of 1, 2, and 4 hr provided the data in Table 1. The regression line of weight difference of dose is estimated by $Y = 1.66 + 1.46X$, where Y is the estimated lung weight difference (g) of a guinea pig surviving exposure to agent, and X is the period of exposure (hr). The slope of this regression line is 1.46; that is, about 1.5 g lung weight change per hour exposure is significantly different from zero ($p = 0.05$). The standard error of the slope b is $\text{S.E.}_b = \pm 0.4$.

References and Notes

1. Rahn's method of preparation of many lungs of many species are well known to those familiar with department of physiology at Rochester. It has so far not been in print.
2. W. Prandtl and K. Sennewald, *Ber.* **62B**, 1754 (1929).

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Every honest researcher I know admits he's just a professional amateur. He's doing whatever he's doing for the first time. That makes him an amateur. He has sense enough to know that he's going to have a lot of trouble, so that makes him a professional.— CHARLES F. KETTERING.