ative with tactual percussion; the slowest, of the C group (2 to 5 m/sec), were related to burning; those generated by pricking had a velocity 20 to 30 m/sec; and finally, those due to irritative slight contacts which Zotterman thought might induce itching had a velocity of 8 to 17 m/sec and were related to the A group, δ type fibers.

As I pointed out (*10*), it appears to me that there is remarkable agreement between these data and the velocities found in man through measurement of reaction times: 40 m/sec for touch, 4.5 m/sec for burning, 16 m/sec for pricking, and about 12 m/sec for pinching.

This finding that there is dissociation of afferent systems for painful excitations of the skin has been shown to be in agreement with numerous other data, some of which were reported by G. H. Bishop and W. L. Landau (*11*); it can be considered to be a definitively established fact (*12*).

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2 January 1959

Due to limitation of space, I shall limit my discussion to the reaction-time studies which are assumed to prove the duality (or even plurality) of cutaneous pain systems.

I have stated (*1*) that none of the studies of reaction time meets the minimum requirements for reliable results. I repeat, none of them do—not excluding the study of Lele, Sinclair, and Weddell (*2*). But the work of Lele *et al.* was technically superior to most, and it did show that, even in studies of the sense of touch (far easier to deal with than pain), variability of results is the rule. The conclusion I draw is that more than ordinary caution is required to demonstrate a reliable difference in reaction time between two areas.

In the study of pain, proper control of stimulus has been a vexing problem. With needle stimuli, even when properly applied, there is a variation in time lag before stimulation because of the appreciable amount of time necessary for the needle to penetrate to its maximum depth (*3*), and because of the varying depth of the receptors. With heat stimuli, the time lag is both greater and more variable because of variation in the thickness and character of the epithelium in different areas of the body (*4*). And the difficulties involved in precise control of heat stimuli are many (see *5*).

The study which Piéron cites as definitely establishing the dissociation of pain systems is his own (*6*). In this study, on one test subject, a needle was used to produce pricking pain, with pressure of 15 g on the temple, 25 g on the wrist, and 25 g on the ankle. As far as one can ascertain from the report, 24 trials were made at each point. There is no information about pretraining on this type of response (and in any event, the learning curve for reaction-time data does not level off until at least the 100th trial) or about the subject's "coherence" —or, indeed, about how much he knew about what the experimenter expected to find. The intensities used are greatly above those of the pain threshold, and a statement that they were approximately equated for the three areas is not convincing, in the absence of experimental data, in view of the great variability in pain threshold of various pain spots (*7*) and of the extreme difficulty of making that type of psychophysical judgment. Further, there is no measure of the significance of the differences in reaction time used to calculate conduction velocities in nerves.

In the same study, burning pain was produced by application of a metal container filled with water at 70°C (or 60°C?) to the forehead and the back of the hand (four trials each). Since this stimulus gave reaction times for the foot which were too long to be "useful," the difference in reaction time between hand and foot was determined by plunging them into a hot (60°C) water bath (11 and 4 trials, respectively). In neither case is the stimulus constant over time, nor does it bear any observable relation to the threshold for heat pain. In the latter case, even the areas vary. Heat stimuli, to be even moderately controlled, must be constantly monitored, and even then changes or differences in blood flow, color of skin, and chemical changes within the tissues (particularly upon repetition) may render the control superficial.

Heat of this order penetrates tissues more slowly than a needle, and the slower reaction times to heat pain are certainly correlated with the time lag between application of the stimulus to the surface of the skin and the stimulation of the underlying receptors. Furthermore, the differences in the epithelium in various regions of the body would lead one to expect a greater time difference in the response to heat of forehead, hand, and foot than in the response of these areas to suprathreshold stimuli produced by a needle.

McKenna (*8*) found that neither surface temperature nor increase in surface temperature is critical for stimulation of pain by heat, but, rather, that the important factor is either the critical temperature at the receptor or the temperature difference between receptor level and deeper fibers. Thus, the depth and thermal characteristics of the epithelium would seem to be important determinants of absolute reaction time, as well as of the differences in reaction time between various regions of the body.

Until a slow pain ("subjective" because perceived) in the absence of the afore-mentioned artifacts can be demonstrated, there exists no body of data to be related to the physiological data regarding cutaneous C-fiber function.

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27 March 1959

Separation of Hydrogen Isotopes by Gas-Solid Chromatography

Abstract. Conditions are described for the chromatographic analysis of mixtures of H_2, HT, and T_2 on a "molecular sieve" column. This technique may find valuable applications in various kinetic investigations.

Isotope effects in gas chromatography have been observed previously (*1*). We have found that this phenomenon can be used to analyze mixtures of H_2, HT, and T_2. Samples were prepared by

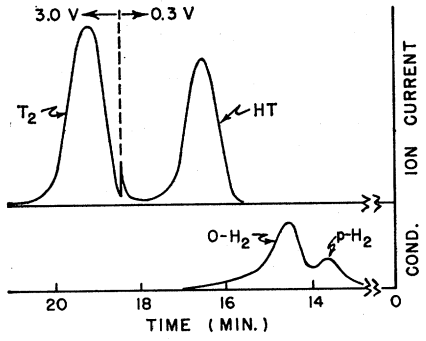

Fig. 1. Separation of ortho- and para- H_2, HT, and T_2 by gas-solid partition chromatography.

sparking mixtures of about 1 percent T_2 in H_2 with a Tesla coil. They were passed through a 6.1-m molecular sieve column with helium carrier gas. The column was kept at −160°C with a bath of liquid methane containing about 10 percent ethane. The gas chromatograph was modified by the addition of a 10-cm³ ionization chamber in series with the thermal conductivity cell (*2*). Both detectors were kept at 30°C. The chemical peaks (thermal conductivity) and radiochemical peaks (ion current) were recorded on synchronized recording potentiometers. The results are depicted in Fig. 1. Hydrogen showed two peaks with relative heights of 3 to 1, corresponding to ortho- and para-hydrogen (*3*). A sample of pure para-hydrogen gave only one peak at retention time of 13.5 minutes. Prolonged treatment of a sample with a Tesla coil increased the HT peak and decreased the T_2 peak. When the sample was placed on activated uranium (*4*), then taken out, the peak identified as T_2 disappeared.

One of the easily controlled variables in the analysis was the flow rate of the carrier gas. In Fig. 2, the heights equivalent to a theoretical plate (H.E.T.P.) for HT and T_2 are plotted against flow rate of carrier gas (*5*). From this figure, it is concluded that a flow rate of about 10 cm/sec gives maximum separation. The number of theoretical plates for HT and T_2 were then 4060 and 4580, respectively.

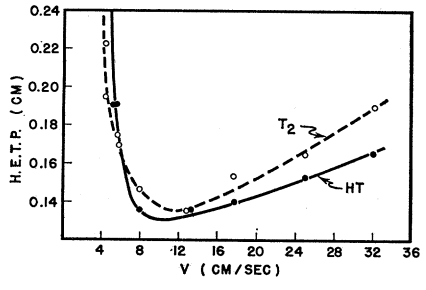

Fig. 2. H.E.T.P. (cm) for HT and T_2 versus flow rate of helium carrier gas (cm/sec).

The results reported here can be utilized for investigating the kinetics of some interesting systems: for example, the radiolysis of $T_2 + H_2$ mixtures and the formation of HT in Wilzbach labeling (*6*).

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12 January 1959

Electrophoretic Method for Desalting Amino Acids

Abstract. A solution to be desalted is placed on a paper strip along which an ammonium formate buffer gradient has been established. Application of a potential brings about migration of amino acids to their isoelectric *p*H's and removal of salt ions. The strip is eluted with water, and the eluate is freed of ammonium formate by vacuum sublimation at 40°C.

Salts in amino acid preparations (for example, protein hydrolyzates or tissue extracts, to be analyzed chromatographically) are objectionable (*1, 2*). Several desalting procedures have been developed but, according to Block *et al.* (*1*), not one is completely satisfactory.

The need for a simple general method of desalting micro quantities of amino acids and peptides from protein hydrolyzates led to the development of an electrophoretic procedure that takes advantage of the volatility of ammonium formate [previously exploited in ion-exchange chromatography of amino acids by Hirs *et al.* (*3*)]. The sample to be desalted is subjected to electrophoresis on a paper strip along which a *p*H gradient is established by use of ammonium formate buffer. The amino acids migrate to the region of their isoelectric *p*H at or near the midpoint of the strip, the unwanted salt ions move to the solutions around the electrodes, and ammonium formate is left as the only electrolyte on the strip. After electrophoresis, the strip is eluted with water, and the eluate is vacuum dried at 40°C. The ammonium formate sublimes, and the desalted hydrolyzate remains as a residue.

The method was tested as follows with single-strip Durrum-type paper electrophoresis cells (*1*, p. 511; *4*): A 1- by 10-in. strip of Whatman No. 1 paper, held vertically, was washed by allowing 200 ml of water to flow down through it (as in descending chromatography). The ends of the dried strip were placed in the electrode compartments with ammonium formate buffers of 0.1 ionic strength at *p*H's above and below the isoelectric points of any amino acids present [for example, above 10.8 and below 2.3 if both arginine (*pI* 10.8) and aspartic acid (*pI* 2.3) are present]. The paper was supported at its midpoint by a horizontal glass rod (3 mm in diameter) rubbed with silicone stopcock grease (*1*, p. 512). The sample of solution to be desalted (usually 10 to 30 μl) was applied at the midpoint of the strip, a glass cover was placed over the strip, and the buffer solutions were allowed to migrate up the strip to complete the liquid junction. A potential of about 50 v per cell was applied overnight, the electrode in the low *p*H buffer being positive. After electrophoresis, the ends of the strip were cut off just above the buffer vessels, the strip was allowed to dry at room temperature, and the amino acids were eluted by allowing 20 ml of water to flow down through the vertically suspended strip. The eluate was vacuum-dried for at least 16 hours at 40°C (*5*). The possibility of scaling up the quantity of sample is indicated by the recovery of 0.98 g of glycine from a solution of 1.00 g of glycine in 5 ml of 0.1*M* KCl on a single large sheet of Whatman No. 1 paper in the Spinco model R apparatus.

Figure 1 shows two-dimensional chromatograms of 20-μl samples of amino acid solutions in 1*N* NaCl containing

Table 1. Data on recovery and salt removal for 80 μl of 1 percent DL-phenylalanine in 1*N* NaCl. The values shown in parentheses were obtained before desalting.

Absorbance (at 280 mμ)	Recovery of amino acid (%)*	NaCl (mg)	NaCl remaining (%)*
	Experiment 1		
0.161 (0.137)	95	0.12 (4.68)	1.1
	Experiment 2		
0.170 (0.137)	102	0.08 (4.68)	0.2
	Experiment 3†		
0.031 (0)		0.07 (0)	

* After correction for blank.
† Blank (no sample added to filter paper strip).