References and Notes

1. B. D. Burns and W. D. M. Paton, *J. Physiol. (London)* **115**, 41 (1951).
2. S. Thesleff, *Acta physiol. scand.* **34**, 218 (1955).
3. S. Ochs and A. K. Mukherjee, *Am. J. Physiol.* **196**, 1191 (1959).
4. S. Ochs and B. Annis, in preparation.
5. H. P. Jenerick and R. W. Gerard, *J. Cellular Comp. Physiol.* **42**, 79 (1953).
6. A. M. Shanes, *Pharmacol. Rev.* **10**, 59 (1958).

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Deuterium Analysis—a Simple and Precise Method

Abstract. By means of reaction with calcium hydride in a generator of simple design, the water samples are converted into H_2 and HD. With hydrogen as carrier gas, the greater thermal conductivity of HD produces a peak whose size is linearly related to the deuterium content of the original water.

Described below and depicted in Fig. 1 is a simple, inexpensive apparatus which analyzes water samples (without special purification) for deuterium content with a precision that is generally better than the mass-spectrometer and falling-drop procedures currently in use (*1*).

The mixture of HOH, DOD, and HOD (about 0.1 ml) contained in reservoir bulb *B* (Fig. 1) is allowed to drop slowly onto granules of calcium hydride contained in cartridge *C* suspended in evacuated tube *A*. This generates a mixture of only H_2 and HD and since the separation factor (*2*, *3*) for the reaction of mixed waters with calcium hydride is nearly 1.00, the HD content of the gas corresponds almost exactly to the deuterium content of the water sample. Three-way stopcock *D* connects the gas generator to double-arm manometer *E* and the gas sampler of the gas chromatography equipment *F*. After evacuation of the manometer-sampler system, the system is sealed off with stopcock *G*, through which the system is connected to a vacuum pump. The gas mixture from *A* is now introduced through *D* into the evacuated sampler and manometer and the pressure throughout is adjusted to atmospheric pressure by manipulation of the leveling bulbs. The mixed gases are now released into the carrier stream of the chromatograph.

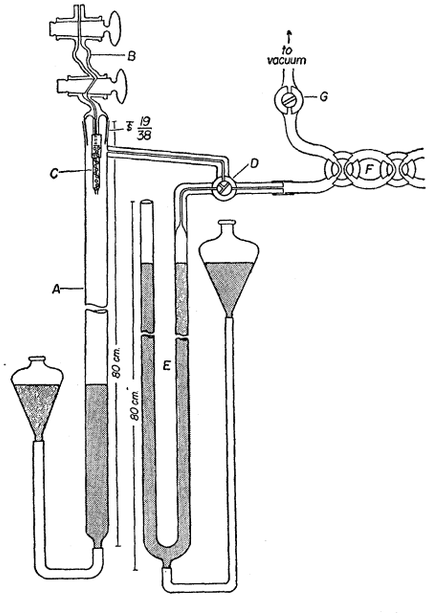

Fig. 1. Apparatus used to convert deuterium into H_2 and HD.

No attempt is made to separate hydrogen from HD (*4*). Instead, the recording katharometer of the chromatograph is used to measure the well-known (*1*) difference between the thermal conductivity of HD and that of hydrogen, the function of the column (Burrell High Activity Charcoal) being to separate volatile impurities from the hydrogen isotopes. Hydrogen is used as the carrier gas so that the size of the peak traced by the recorder depends only on the quantity of HD in the gas sample. This gives the method great sensitivity and we have detected D_2O at twice the background level (0.017 mole percent).

From 0 to 10 mole percent deuterium—the region of greatest interest for tracer studies—the relation of peak height to mole percent deuterium in the original water is strictly linear (*3*) (Fig. 2). Series of replica samples analyzed during the same day often agree within an estimated standard deviation of 0.3 (relative) percent while replicas analyzed on different days usually have a standard deviation of 0.5 to 1.0 percent, depending on deuterium content. Thus our results compare favorably with conventional methods which have a "precision" of 0.5 to 3 percent (*1*). We find that the calibration line holds within the above error over the life of a tank of hydrogen (about a week), if the hydrogen flow is left undisturbed night and day and correction is made for change in atmospheric pressure. Since the calibration curve is a straight line passing through the origin (by least squares), a single run with a standard solution serves for recalibration. Analytical precision may be improved by recalibrating immediately after running an unknown using a standard solution of nearly the same deuterium content. We are currently developing the sensitivity and precision of our method and expect that both may be improved considerably.

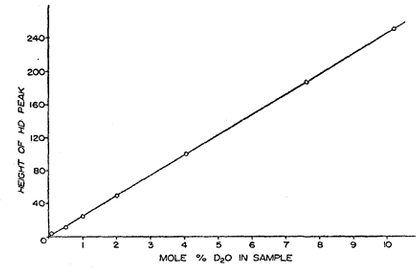

Fig. 2. Each peak height shown on this curve is an average taken from the heights of 6 to 12 curves obtained from 2 to 4 samples run on 2 to 4 different days. They have been corrected for changes in barometric pressure. Peak heights for this system are more reproducible than peak areas measured with a planimeter.

A standard Burrell Kromotog K-2 was used with a hydrogen flow of 40 ml/min, a cell current of 310 ma and a 2½-m column, at room temperature. A 20-ml gas sampler thermostatted at 50°C was employed in order to get large peaks for the less concentrated heavy water solutions. Better results for solutions with more than 2 mole percent of heavy water can be obtained by using a smaller sampler. Indeed, a great advantage of the method is the flexibility made possible by the use of different-sized gas sampler chambers for different HD concentrations, which permits the use of high recorder sensitivity and high precision over the whole H_2–HD range from 0 to 100 percent except at the extreme ends. The standard solutions used for the calibration line were prepared from deaerated, distilled water and deuterium oxide (General Dynamics, 99.9 percent). The exact deuterium content of every sample was established with a 25-ml pyknometer (*1*) at 25°C.

Reproducibility depends mainly on the freshness of the calcium hydride surface (Metal Hydrides, Inc.), the constancy of carrier gas flow, and the scrupulous avoidance of leaks during evacuation. The latter are easily detected both with the manometer and by the appearance of air peaks on the recording (*5*).

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References and Notes

1. I Kirshenbaum, *Physical Properties and Analysis of Heavy Water* (McGraw-Hill, New York, 1951).
2. E. D. Hughes, C. K. Ingold, C. L. Wilson, *J. Chem. Soc.* **1934**, 493 (1934).
3. Preliminary studies indicate that the relation is perfectly linear, over the whole range, to concentrated solutions of D_2O.
4. Several research groups [E. Glueckauf and G. P. Kitt, *Vapor Phase Chromatography*, D. H. Desty, Ed. (Academic Press, New York, 1957); S. Ohkoshi, S. Tenma, Y. Fujita, T. Kwan, *Bull. Chem. Soc. Japan* **31**, 772 (1958); W. R. Moore and H. R. Ward, *J. Am. Chem.*

Soc. **80**, 2909 (1958); C. O. Thomas and H. A. Smith, *J. Phys. Chem.* **63**, 427 (1959)] have approached G. L. C. deuterium analysis by separation of the isotopes. Since this involves partitioning the deuterium under two peaks rather than converting it to one measurable species, and usually requires conditioning of the stationary phase between samples, we feel that our method is inherently better, precisely because we do *not* separate the gases.

5. We thank the National Science Foundation for supporting this work, and Mr. Lloyd Guild of the Burrell Corp. for advice. This report was presented 2 March 1960 at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy.

23 February 1960

Inheritance of a Serum Protein in Swine

Abstract. A study of polymorphism of the starch-gel electrophoretic pattern for one of the blood serum proteins in swine (tentatively designated protein B) reveals that it is controlled by a single pair of alleles exhibiting partial dominance. *BB* genotypes appear to have twice the amount of protein B that the *Bb* genotype has, while *bb* genotypes show no evidence of the protein. Present indications are that the Yorkshire and Landrace breeds differ in the frequency of these genes.

Inherited variations of serum proteins in human beings have been studied by Smithies and Walker (*1*) and in cattle by Smithies and Hickman (*2*) and by Ashton (*3*). Starch-gel electrophoresis of pig serum was reported by Ashton (*4*) without any genetic study of the polymorphisms observed.

In this investigation, serum samples from all the parents involved in 100 litters and from random samples of progeny in these litters were subjected to vertical starch-gel electrophoresis (*5*), and the resulting electrophoretic patterns were related to pedigree information in order to determine whether the polymorphism observed was under genetic control. Blood was collected from the ear veins on sows and boars, and by cardiac puncture from 28-day-old pigs. Serum samples were stored at −25°C.

The vertical starch-gel electrophoresis technique used in this study was essentially that of Smithies (*6*, *7*), with the following differences. The buffer was made up with 125 ml of 0.2*M* tris(hydroxymethyl)aminomethane (Fisher) to which was added 62.5 ml of 0.1*N* HCl and 312.5 ml of distilled water. Seventy grams of Starch-Hydrolysed (Connaught Medical Laboratories, Toronto) and 500 ml of Tris buffer were used to prepare each gel. The electrophoresis was carried out at a voltage gradient of 5 volt/cm for 17 hours.

After staining and destaining, gels were photographed on 4 by 5 inch Kodak Verichrome Pan film with a Wratten filter F. Contact prints were used as a permanent record of each gel.

Figure 1 illustrates the types of electrophoretic patterns which were observed for the protein tentatively designated as protein B.

The analysis of the data gathered to date with respect to protein B is presented in Table 1. As indicated by the probability values, χ^2 tests support the hypothesis that phenotypes I, II, and III result from genotypes *BB*, *Bb*, and *bb*, respectively. The poorest fit was noted for progeny from the II × I matings. This suggests the possibility of a semilethal interaction between a *Bb* genotype in the progeny and a *BB* genotype in the dam.

In order to examine this possibility further, a heterogeneity χ^2 test was carried out for the progeny distributions resulting from the I × II and II × I mating classes. The results ($\chi^2 = 1.371$, $P > .20$) indicated that both mating classes were likely samples from a single population of matings which produces segregation in a 1:1 ratio. Further data will be required to determine whether this deviation has any real significance. When progeny totals from I × II and II × I mating classes are pooled, the fit to a 1:1 ratio is quite reasonable ($\chi^2 = .371$, $P > .50$).

Among the 100 litters involved in this study, 38 were Yorkshire × Yorkshire, 16 were Yorkshire × Landrace, 32 were Landrace × Landrace and 14 were Landrace × Yorkshire (males are identified first). Of particular interest is the distribution of genotypes among sows of each of the breeds (Yorkshire 22 *BB*, 27 *Bb*, 3 *bb;* Landrace 46 *BB*, 2 *Bb*, 0 *bb*).

The application of a χ^2 test for uniformity of genotype distributions among sow herds ($\chi^2 = 33.093$, $P < .01$) indicates that the genotype distributions, and therefore the gene frequencies, are significantly different. This is particularly interesting since the Landrace sows represent samples from 29 different Ontario breeders and the Yorkshire sows are descended from a wide sample of dams and 15 unrelated boars purchased from 12 different Ontario breeders during the past 3 years. The distribution of genotypes among Yorkshire boars involved in this study was 2 *BB*, 3 *Bb*, 0 *bb*, and among Landrace boars, 4 *BB*, 0 *Bb*, 0 *bb*. It is tentatively concluded that the Yorkshire and Landrace breeds differ markedly in frequency of the genes controlling development of serum protein B (*8*).

Table 1. Distribution of observed progeny phenotypes (o) from various mating classes and those expected (e) on the hypothesis that phenotype I = *BB*, phenotype II = *Bb*, and phenotype III = *bb*.

Item	Progeny phenotypes I (*BB*)	II (*Bb*)	III (*bb*)	*P* of χ^2
	Mating class I × I			
o	109			
e	109			
	Mating class I × II			
o	25	26		
				>.80
e	25.5	25.5		
	Mating class I × III			
o		7		
e		7		
	Mating class II × I			
o	34	24		.10–
e	29	29		.20
	Mating class II × II			
o	24	47	27	
				>.80
e	24.5	49	24.5	
	Mating class II × III			
o		3	1	
e		2	2	
	Totals			
o	192	107	28	
				>.80
e	188	112.5	26.5	

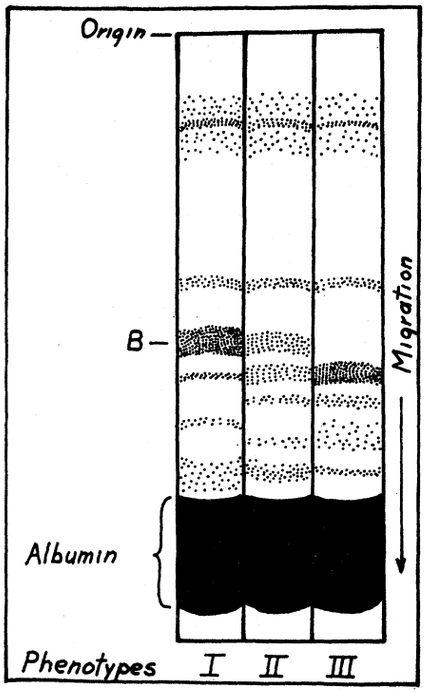

Fig. 1. Diagrammatic illustration of electrophoretic patterns showing the variants observed for protein B. Additional variation to that illustrated has been observed for other proteins.

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References and Notes

1. O. Smithies and N. F. Walker, *Nature* **176**, 1265 (1955); **178**, 694 (1956).
2. O. Smithies and C. G. Hickman, *Genetics* **43**, 374 (1958).
3. G. C. Ashton, *Nature* **180**, 917 (1957).
4. ———, *ibid.* **179**, 824 (1957).
5. I wish to thank W. Zeitz for his technical assistance in the electrophoresis work involved in this study.
6. O. Smithies, *Biochem. J.* **61**, 629 (1955); **71**, 585 (1959).
7. I wish to express my thanks to O. Smithies for his interest and advice on vertical starch-gel electrophoresis.
8. This report is contribution No. 16, Animal Research Institute, Research Branch, Canada Department of Agriculture.

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