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The Direction of Flow in the Blood Vessels of the Infundibular Stalk

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Recent work, especially that of G. W. Harris (*1*), has drawn attention to a possible neurohumoral relay between the hypothalamus and the anterior lobe of the hypophysis. The neural link in the chain consists of nerve fibers from the hypothalamus to the median eminence and infundibular stem, where the humoral substance originates and is transmitted to the adenohypophysis via the hypophyseal-portal blood vessels.

The direction of blood flow in the vessels of the stalk forms an important part of the theory. Wislocki (*2*), as well as Green and Harris (*3*), suggested that the direction of flow was from the median eminence and infundibular stem to the adenohypophysis. This is in contradistinction to the report of Popa and Fielding (*4*), in the original description of the hypophyseal-portal vessels, stating the blood flow was from the anterior hypophysis up the stalk to the hypothalamus.

During recent investigations into the anatomy and physiology of the pituitary gland and stalk (*5*), a surgical procedure was developed to expose completely the stalk and rostral portion of the pituitary gland to direct vision. The operative approach was parapharyngeal, and the blood vessels supplying the hypophysis were not disturbed. It occurred to us that with this exposure the direction of flow in the blood vessels of the infundibular stalk could be visualized.

The procedures were carried out on adult albino rats. The region of the infundibular stalk was exposed surgically with the animals under ether anesthesia. By this means the blood vessels of the infundibular stalk were brought into view. The chest was then opened, and a fine glass cannula was introduced into he proximal aorta through the wall of the left ventricle. Less than 5 ml of a 50% aqueous suspension of Higgins waterproof India ink was injected slowly while the infundibular stem blood vessels were viewed with a binocular dissecting microscope. India ink usually appeared in several vessels within a few seconds of the beginning of injection. This occurred before any other vessels or tissues in the operative field became injected. With a just appreciable lag, involving perhaps no more than a second, additional vessels, usually 3 in number, became filled. By varying the quantity of ink injected, as well as the injection pressure, it was possible to fill selectively the first-mentioned vascular channels, or to fill all of them (an average of 6). It was noted repeatedly that the flow of India ink in all these vessels was invariably from the stalk to the body of the hypophysis. The vessels extended from the anterior part of the stalk posteriorly to the pituitary gland, where they ramified. They had the approximate width of a very fine silk thread. One or two of these vessels entered the posterior lobe. The pars distalis did not become colored until the India ink passed down the vessels of the stalk to the gland, nor did the distalis become colored if the stalk was severed just prior to injection. We have therefore been able to substantiate the anatomical observations of Wislocki (*2*) and Green and Harris (*3*) by direct visualization of the flow of India ink in the infundibular stalk vessels.

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A Universal Line Graph for Estimating Percentage Potency in Multidose Assays

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In 1947 the author (*1*) presented in these columns simple formulas for calculating percentage potency in 3- and 4-dose assay procedures, when the log dose-response curves of the unknown and standard materials are both linear and parallel. Later Harte (*2*) demonstrated that each formula was reducible to a single line which, under the conditions of that test, could be used for a rapid graphic determination of the percentage potency. This was a distinct improvement over the use of radial lines employed by Knudsen (*3*), but still left much to be desired, since the position of the line varied with the two parameters: C, the log ratio of the concentration of the unknown to that of the standard, and d, the log interval between the successive doses of both the unknown and standard. Harte, however, avoided the second parameter by the use of a fixed d and established a

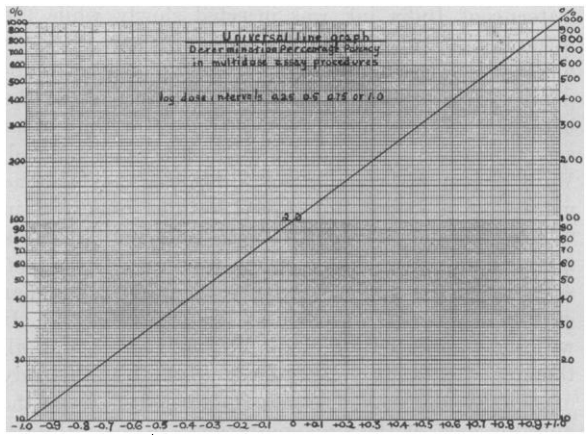

FIG. 1.

family of parallel lines, the position of which was determined by C.

It occurred to the author that if a single line could be drawn that would have universal applicability to all assays in which the requirements of linearity and parallelism were met, it should prove extremely useful. Such a condition of universal applicability within the limits of 10–1,000% would be met if in the basic formula, Percentage potency =[Antilog $2.0 \pm C \pm kdP$],[1] the concentration ratio, C, were omitted and kd were always some simple multiple of 1.0. Then on semilogarithmic paper[2] (Fig. 1) a line drawn diagonally from the 1 in the lower left-hand corner to the 1 in the upper right-hand corner would pass through 2.0, i.e., 100% at the central perpendicular line zero. The heavy lines to the right of zero on the base line would then be labeled + 0.1, + 0.2, + 0.3 . . . . . + 1.0, and, similarly, reading from right to left, those at the left of zero would be labeled − 0.1, − 0.2 . . . . . to − 1.0. The figures on the perpendicular logarithmically divided lines in the right and left margins would in the lower cycle correspond to 10, 20, 30 . . . . . 100%, and those in the upper cycle to 100, 200 . . . . . 1,000%.

To determine the percentage potency graphically one follows 6 simple steps: (1) the replicate observations for each dose level of standard and unknown are summed; (2) corresponding levels of U and S values are inserted in the formula for P for the number of doses employed (Table 1); (3) the arithmetic processes involved in this formula are carried out; (4) the P value found is treated as indicated on the right-hand side of the table according to the dilution employed; (5) the figure obtained from the multiplication and/or division of the original P is used to enter the base line to the right or left of zero, depending upon the sign; and (6) by following the entry perpendicularly to its intersection with the line and then horizontally to the right- or left-hand margin, one is enabled to read the percentage potency directly. Although a family of parallel lines based upon different C values can be established as Harte suggested, the author feels that the ratio of concentration of unknown to standard can best be handled by a separate multiplication or division of the percentage value obtained graphically.

TABLE 1

USE OF P TO OBTAIN PERCENTAGE POTENCY ON UNIVERSAL LINE GRAPH

No. doses/ assay	k*	P	Dilution			
			1: 1.78	1: 3.15 or 1: 3.2	1: 5.6	1: 10
			Enter base line of graph at			
2	1	$\frac{(U_2+U_1)-(S_2+S_1)}{(U_2+S_2)-(U_1+S_1)}$	$P_{/4}$	$P_{/2}$	$3P_{/4}$	P
3	4/3	$\frac{(U_3+U_2+U_1)-(S_2+S_2+S_1)}{(U_3+S_3)-(U_1+S_1)}$	$P_{/3}$	$2P_{/3}$	P	$4P_{/3}$
4	5	$\frac{(U_4+U_3+U_2+U_1)-(S_4+S_3+S_2+S_1)}{[2(U_4+S_4)+(U_3+S_3)]-[2(U_1+S_1)+(U_2+S_2)]}$	$5P_{/4}$	$5P_{/2}$	$15P_{/4}$	$5P$
5	4	$\frac{(U_5+U_4+U_3+U_2+U_1)-(S_5+S_4+S_3+S_2+S_1)}{[2(U_5+S_5)+(U_4+S_4)]-[2(U_1+S_1)+(U_2+S_2)]}$	P	$2P$	$3P$	$4P$
7	8	$\frac{(U_7+U_6+U_5+U_4+U_3+U_2+U_1)-(S_7+S_6+S_5+S_4+S_3+S_2+S_1)}{[3U_7+S_7)+2(U_6+S_6)+(U_5+S_5)]-[3(U_1+S_1)+2(U_2+S_2)+(U_3+S_3)]}$	$2P$	$4P$	$6P$	$8P$

* These values may be inserted in basic formula, percentage potency = Antilog $[2 \pm C + kdP]$, to obtain potency with number of doses used. For example, in the 5-dose assay formula, as follows: Percentage potency = Antilog $[2.0 \pm C + 4dP_5]$. The use of this formula, however, necessitates the finding of the antilog from tables of logarithms.

d = log dose interval. U and S refer to unknown and standard, respectively, and subscripts represent dose levels, larger subscripts denoting greater concentrations.

[1] P is used in the sense in which Harte employed it and represents the fraction referred to in Sherwood's original paper, P_2 being P for a 2-dose assay, and P_5 that of a 5-dose assay (Table 1); 2 is the logarithm of 100%; k is a parameter which varies according to the number of doses used in the standard and the unknown; and d is the log-dose interval.

[2] Semilogarithmic paper, 2-c, short side × 20 to the in., K & E No. 359 64, is used.

It will be noted that in Table 1, P for the 6-dose assay is omitted. This is because k for the 6-dose assay

TABLE 2

EFFECT ON PERCENTAGE POTENCY—PRECISE DILUTIONS VS. SIMPLE DILUTIONS

Precise dilution	Log-dose interval	Simple dilution	Log-dose interval	Percentages obtained by precise dilution			
				10%	66.67%	150%	1,000%
1:1.7783	0.25000	1: 1.78	0.25042	9.96	66.62	150.10	1,003.88
1: 3.1623	0.50000	1: 3.15	0.49831	10.08	66.76	149.80	992.20
		1: 3.2	0.50515	9.77	66.39	150.63	1,024.00
1: 5.6234	0.75000	1: 5.6	0.74896	10.06	66.73	149.82	994.45

Calculations were made by substitution in the 2-dose assay formula in which $\pm C$—i.e., log concentration relationship between standard and unknown—has been omitted. Formula then reads Percentage potency = Antilog $[2.0 \pm dP_2]$. $2.0 = \log 100$, $d = \log$ successive dose intervals, and $P_2 = \dfrac{(U_2 + U_1) - (S_2 + S_1)}{(U_2 + S_2) - (U_1 + S_1)}$

With dilution of 1 : 1.7783 log-dose interval = 0.25000. Log of 10% = 1.0; hence, by substitution, formula becomes Percentage potency = Antilog $2 + 0.25000(-4)$, whereas when 1 : 1.78 used, log-dose interval = 0.25042, and formula reads Percentage potency = Antilog $2 + 0.25042(-4)$, or Antilog 0.99832 = 9.9613%.

is 35/3, and the use of such a figure is inefficient. Although it is not very likely that the 7-dose assay would be used routinely, its P was included for the sake of completeness. It is possible that one might wish to include a larger number of dose levels on an unknown, in which case if necessary one could discard some levels at either or both ends if the limit of linearity of the dose-response curve was exceeded. Percentage potency would then be calculated on the doses of unknown remaining plus an equal number of corresponding doses of the standard.

Table 2 gives a comparison of results obtained when simple dilutions of 1:1.78, 1:3.15 or 1:3.2, and 1:5.6 replace the precise dilutions of 1:1.7783, 1:3.1623, and 1:5.6234. It should be noted that at 100% the error is zero. Since the errors at the ends of the curve are so slight in comparison with the errors inherent to the assay methods, the use of the simpler dilutions in routine tests is suggested.

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Potassium Excretion in Rats

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There is evidence that potassium is filtered through the glomeruli and reabsorbed partially by the tubules, in normal man (*1, 2*), dog (*3*), and rat (*4*). It has recently been suggested, however, that in certain circumstances potassium may also be secreted by the tubules; this was shown in anesthetized dogs during "forced osmotic diuresis" with urea (*5*), in unanesthetized dogs after administration of salyrgan or intravenous injection of a hypertonic solution of potassium chloride (*6*), and in human beings suffering from severe renal insufficiency (*7*).

When investigating the mechanism of sodium and potassium excretion in rats (*4*) a few cases in which the potassium clearances were found to be higher than that of inulin were deliberately discarded: they were (*a*) animals which in spite of the administration of 5% of their body weight of water had an abnormally low urine flow, probably as a result of accidental dehydration, and (*b*) rats in which the rate of urine flow was so high that a faulty measurement of the urine volume was assumed. It is known that under both circumstances there is a release of intracellular potassium resulting in an increase of the plasma potassium concentration. In two rats, where the urine flow amounted to .0010 and .0015 ml/100 g/min only, the ratio potassium clearance/inulin clearance was 1.1 and 1.3, respectively; similar results were found in two other animals with an extremely high rate of urine flow: .1070 and .1062 ml/100 g/min. In these cases, values for T_K (= amount of K reabsorbed expressed as percentage of that filtered [*4*]) were negative, indicating that some potassium had been secreted by the tubules and added to the glomerular filtrate.

These findings suggested to Heller (*8*) an interesting interpretation of some previous results (*9*): Heller had found that after 24 hr of dehydration the amount of potassium excreted in the urine was increased by 35% in adult rats, but decreased by 54% in newborn animals. As no evidence of a significant decrease in the glomerular filtration rate could be shown in these adult dehydrated animals, it would seem likely, from the above results, that the enhanced potassium excretion was the result of a tubular secretion of that ion. Such secretion, however, did not seem to occur in newborn rats, in which the depression of glomerular filtration seems to be the principal factor regulating the renal excretion of potassium (*2*).

This discrepancy between the tubular function of adult and newborn rats is in line with results of two independent series of investigations carried out recently in this department. It could be shown that the urine flow of newborn rats remains unaffected after the administration of either diuretics (Dicker, unpublished) or vasopressin (Heller, unpublished). The lack of sensitivity of the tubules of newborn rats to pharmacological and physiological stimuli (*10*) is thus