phylactic agents until a satisfactory technic for the evaluation of protective antibody becomes available.

The complement-fixation test should also prove useful in determining the presence or absence of virus in attempts to propagate the latter in tissue cultures, the developing chick embryo or in material obtained from animals hitherto regarded as insusceptible.

Finally, it is not impossible that when appropriate surveys are completed on the incidence of positive and negative reactions in various age groups and in groups of persons giving positive and negative histories of the disease, the complement-fixation test may be found to be of value in the detection of the susceptible individual.

It is evident, however, that the extensive application of the test will depend upon the discovery of a more readily available and cheaper source of antigen.

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A Method for Demonstrating an Antidiuretic Action of Minute Amounts of Pitressin: Statistical Analysis of Results.

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Using single rats rendered diuretic by the administration of water and alcohol, it has been possible to detect the antidiuretic action of as little as 0.02 milliunit or 20 microunits of pitressin given intravenously. The method is conservative of time and material. As an assay technic, it is at least as accurate as others heretofore reported.

Healthy male rats, of unselected stock, weighing 180-220 g were used. They were fed a diet of dog chow cubes, with biweekly additions of fresh vegetables. Prior to the test, they were starved for from 12-18 hours, but water was allowed *ad libitum*. At the time collections of urine were begun, the environmental temperature was brought to 29-30°C, and was maintained constant by the use of an electric reflector heater. Solutions to be administered by gavage were warmed to 40°C.

All containers used for handling pitressin had been carefully washed in sodium hypochlorite solution 5% (commercial), rinsed with hot and cold tap water and distilled water, and dried with alcohol and ether. Ampoules from a single lot of pitressin (No. 161) were

used. A convenient dilution was 1 part pitressin in 200,000 parts of physiological salt solution, where 1 cc = 0.1 μU or 100 microunits. Dilutions were made fresh for each day's experiment and were kept in the refrigerator. Samples removed for injection were used immediately. All injections were flushed through with 0.1 cc of physiological salt solution. The volume of fluid injected did not exceed 0.5 cc per dose.

An initial volume of alcohol-water solution was given by gavage, corresponding to 5% of the rat's weight. This serves as sufficient sedative. The alcohol-water solution contained 12% of 95% alcohol. Thirty minutes later, 3% of the rat's weight of warm tap water was given by gavage. The animal was then fixed securely in the supine position. An intravenous needle (23 gauge, 1½ inches long) was introduced into the femoral vein, after incising the skin, and was clamped in place with its stilet inserted. The glass-tipped connection of a rubber cystostomy tube of 1/16 inch bore was tied into the urinary bladder through a suprapubic wound, after which the bladder was gently replaced and the wound was closed by a single suture. A ligature was tied to occlude the urethra. The collecting cylinder, graduated to 0.1 cc, was set on a stand allowing vertical movement, so that the outlet of the cystostomy tube remained approximately 7 cm below the animal's bladder throughout the experiment.

Measurements of cumulative urine volumes were made at about 10-minute intervals and were plotted on a graph, until three readings indicated that a steady rate of flow, in the range of 3.1-8.1 cc per hour, had been attained. This was usually from 90 to 120 minutes after the first gavage. The first injection of pitressin was then given. Readings were made at 4-minute intervals during the period of antidiuresis.

By subtracting the volume of urine secreted in the 20 minutes subsequent to injection, from that for the same period just prior to injection, a measure of the antidiuretic response (R) was obtained. A maximum of 4 injections was considered to be a feasible limit for a single animal. The interval between injections was 15-50 minutes.

Results. In Table I the effects of 53 injections of pitressin, utilizing 25 animals, are summarized. The series of means of (R) for each (log D) in the 53 observations are given in Table I and plotted as the dots in Fig. 1. It is evident that the relation between mean response and log dose is essentially linear over the range investigated.

TABLE I.
Dose Response Averages in 53 Injections.

Dose micro-units	20	40	80	100	160	200	320
Log Dose	1.30	1.60	1.90	2.00	2.20	2.30	2.51
Mean of R—cc	0.20	0.52	0.93	0.85	1.28	1.25	1.44
Variance of Mean	.0060	.0060	.0112	.0196	.0087	.0393	.0064
Number of observations	13	13	7	4	9	2	5

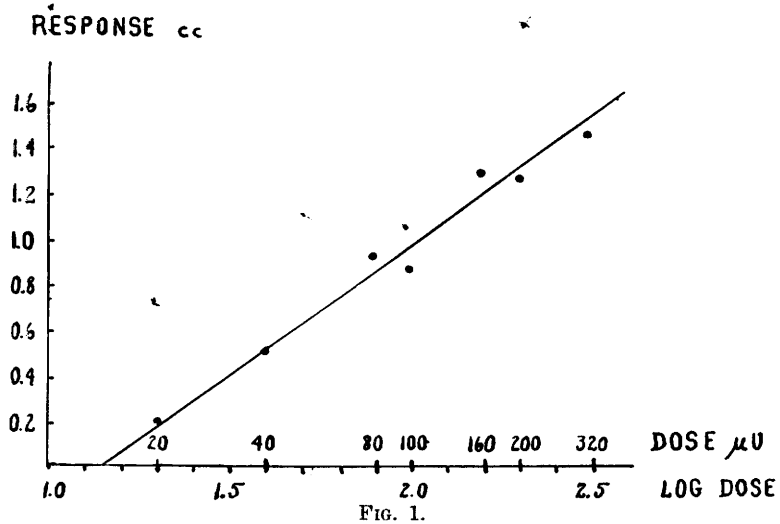

FIG. 1.
Pitressin Antidiuresis. Illustrating the observed linear relation between log dose and antidiuretic response.

In the individual injections the variation of response-dose relation is high, with a standard deviation of (log D) from the expectancy of .28 (variance .08), indicating that 95% of the individual injections tend to give (log D) within ±.56 of the value predicted by the regression line. For Burn's method,[1] using his published data, the corresponding variance is .14 as compared with our .08; this difference is not statistically significant.

The regression of (log D) against (R) was calculated, weighting each mean by the reciprocal of its variance according to the usual statistical method. The resulting equation, for our data, is:

Expected (log D) = 1.137 + .90 R. This equation is shown graphically as the diagonal line in Fig. 1, the dots being the means of (R) for each dose.

Method of Assay. For assaying pitressin, or for comparing other antidiuretic substances with pitressin, it is necessary to evaluate both the potency of the particular lot of pitressin used as compared with that of our lot, and the variability in the animals' responses to iden-

[1] Burn, J. H., *Quart. J. Pharmacy and Pharmacol.*, 1931, **4**, 517.

tical doses of the new pitressin. This can be accomplished without the necessity for performing preliminary tests using pitressin alone, provided all injections of pitressin are given prior to administering other antidiuretic substances which might conceivably alter the effect of pitressin.[2] Our results indicate that it makes no important difference whether all injections of each preparation and of standard are given to one animal, or whether each preparation and standard is given to each of several animals. The order in which various doses are given should be governed by a method of deliberate randomization. The mean log-response relation must be determined for each lot of pitressin with at least as many injections as are used for the unknown. The slopes of our regression lines were not significantly different for the different rats with which we have worked, indicating the propriety of employing a regression line, or equation derived from averaging the data from all observations with standard pitressin.

Thus in assaying the difference between two preparations, an approximately equal number of doses of each preparation should be given to each of several animals, and in such relative doses that the mean response to each preparation will be approximately the same. Correction of the mean dose-response of one preparation to the mean response level of the other preparation can be performed by a shift parallel to one's average regression line, as illustrated in Fig. 1, or by the use of the corresponding coefficient, which is .90 for our data. The difference in log D for the two preparations at equal responses permits an evaluation of the ratio of potency.

The variance of the difference of (log D), for the two preparations at the same mean response, is the sum of 3 components: the variance of estimate of the mean dose at mean response for each of the 2 preparations, and the variance of the correction to equal response level. Its equation, using our observed variance (.08) for individual injections becomes:

$$\sigma^2 = \frac{.08}{n_1} + \frac{.08}{n_2} + .011\ (R_1 - R_2)^2$$

where n = the number of injections of a given preparation, and .011 is the variance of the regression coefficient of the slope (.90). The last term in the equation approaches 0, if the relative doses are selected so as to give equal mean responses for the two preparations.

The following example illustrates the calculation:

Prep. 1	Prep. 2
$n_1 = 3$	$n_2 = 3$
Mean $R_1 = 1.433$	Mean $R_2 = 0.767$

[2] Silvette, H., *Am. J. Physiol.*, 1941, **131**, 601.

Mean log $D_1 = 2.000$ Mean log $D_2 = 2.204$

Cor. mean log $(D_2) = 2.204 + .90\ (1.433 - .767) = 2.803$

Diff. log $D = 2.803 - 2.000 = 0.803$

$$\text{Var. of estimate of log } D_1 = \frac{.08}{3} = .027$$

$$\text{Var. of estimate of log } D_2 = \frac{.08}{3} = .027$$

Var. of cor. of mean log D_2 to response level $R_1 = .011\ (1.433 - .767)^2 = .005$

Var. of calculated diff. of log (D) at the same R $= .059$

Standard error $= .243$

This assay may be taken to indicate that the potency ratio of the two preparations lies between antilog $.80 \pm 2\ (.24)$ that is, Prep. 1 is between 2 and 20 times as potent as Prep. 2. If similar results had been obtained, using 20, instead of 3 injections, the ratio of potency would have been between 5-8, probably a usefully narrow range for assay work.

Summary. Single rats rendered diuretic by water, and alcohol in sedative dosage, constitute a satisfactory biologic test for the antidiuretic action of pitressin. It is 10 times more sensitive than Walker's[3] test, in which he used the diuretic rabbit; however, the required dose of pitressin per kilogram of animal is essentially the same. As an assay technic, using the same number of injections, ours is at least as reliable as the multiple rat test of Burn,[1] and as other tests available for antidiuretic substances.

[3] Walker, A. M., *Am. J. Physiol.*, 1939, **127**, 519.