RESEARCH PAPERS

AN EXAMINATION OF FACTORS AFFECTING THE PRECISION OF THE ASSAY OF THE OXYTOCIC HORMONE IN POSTERIOR PITUITARY LOBE PREPARATIONS

BY G. A. STEWART

From the Wellcome Biological Control Laboratories, Wellcome Chemical Works, Dartford

Received May 3, 1949

EXCEPT in cases where partial separation of the oxytocic and pressor principles of posterior pituitary lobe have been made, this material is usually assayed by methods principally dependent on the oxytocic hormone. We have recently observed several instances of wide variation in the results obtained with the same sample of posterior pituitary lobe powder when assayed in different laboratories by the guinea-pig uterine method, and felt that a fairly comprehensive investigation of the slightly different techniques used might be of value.

Since the guinea-pig uterine method was originally described by Dale and Laidlaw[1], many workers have introduced minor modifications designed generally to overcome difficulties they have encountered themselves. Kochmann[2], for example, found that guinea-pigs weighing 200 to 250 g. were difficult to obtain in large numbers, and he therefore investigated the conditions under which the uteri from larger guinea-pigs could be used. He discovered that a decrease in the concentration of calcium chloride and an addition of magnesium chloride made possible the use of uteri from pregnant guinea-pigs, the weight of the animal being immaterial. Dale and Laidlaw[1], however, had previously stated that a rapid decline in responses to successive equal doses of posterior pituitary extract was shown when the calcium chloride was reduced to half the concentration in normal Ringer's solution. Burn and Dale[3] reported that any method which involved the suppression of automatic rhythm by the reduction of calcium was unsuitable for their purpose. They insisted on the desirability of stating the composition of the Ringer's solution used when comparative results were presented, because changes in the composition of the physiological salt solution modified the sensitivity of the uterine muscle to pituitary extract, to histamine, and to potassium chloride, in varying degrees, and in different directions. This statement is important since many experimenters have looked for conditions under which the uterine assay may be performed more quickly and cheaply without considering the relative effects of their conditions on the response produced by the different hormones of the posterior pituitary gland. The principal modifications have been directed toward the elimination of the type of rhythmic response, occurring frequently, in which the uterus does not contract when stimulated by posterior pituitary extract, in proportion to the concentration of the drug in the bath.

The Influence of the Composition of the Ringer's Solution

Apparent success in the development of a more reproducible response has followed modifications of the Ringer's solution in which the uterine muscle is bathed. The work of de Jalon[4] showed that by decreasing the amount of calcium chloride in the Ringer's solution to 0·012 per cent. and by increasing the magnesium chloride to 0·029 per cent. responses were obtained in which the contraction was proportional to the concentration of posterior pituitary extract, whilst at the same time the sensitivity was increased. Hsu[5] followed up the work of de Jalon and found that the assay of the oxytocic principle may be greatly improved by the use of 0·045 per cent. of magnesium chloride. He claimed that the muscle response was increased, that the spontaneous alternating rhythm of the uterine muscle could be broken or prevented, and that histamine did not interfere. A. M. Frazer[6], however, was able to show that postlobin-V when measured against postlobin-O using Van Dyke and Hastings'[7] solution (a Ringer solution containing a large quantity of magnesium) had an oxytocic activity four times as great as when measured against postlobin-O in ordinary Ringer-Locke's solution, and that magnesium chloride increased the response of the guinea-pig's uterus to both postlobin-O and postlobin-V, the increase in response to the latter being much greater. This aspect of the assay technique was the first to be studied in these laboratories, since the results obtained in five different laboratories with a single sample of posterior pituitary lobe powder had ranged from 400 to 2,000 units/g., and it was known that there were differences in the saline solutions used throughout these comparative assays.

Methods Employed

The usual isolated guinea-pig uterus preparation was used, the animals being virgin albino guinea-pigs weighing 200 to 250 g. The vessel containing the uterine muscle had a capacity of 20 ml. and no metal was in contact with the muscle or the solution, the uterus being impaled upon a glass spike at the bottom of the bath, and attached to the writing lever by a glass hook and a length of thread. The bath was aerated by compressed air, which we have always found to be satisfactory, instead of by oxygen as normally stipulated. Where normal Ringer's solution is referred to, the formula given by the British Pharmacopœia 1948 has been employed, and the modifications of salts to be described have been made to this basic solution. All records have been made using a 2× magnification by means of a light aluminium lever with frontal writing point, and a kymograph surface speed of 1 mm. per minute. Throughout the following experiments, only the magnesium chloride concentration has been modified, and the effect has been observed with mixtures made up from pitocin and pitressin (the purified oxytocic and pressor fractions prepared by Parke Davis and Co.) and with pitocin and pitressin alone. The dose/response relationship was determined for the sub-

maximal uterine responses to two different concentrations of the particular hormone, and the effect of adding different amounts of magnesium chloride solution prior to the addition of the hormones was examined; a section of an experiment is exemplified in Figure 1. The results were

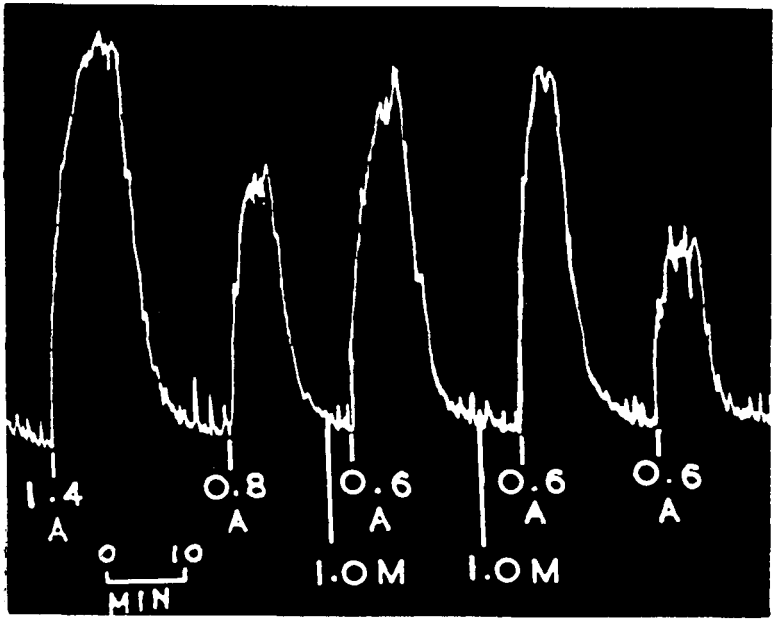

FIG. 1.—The influence of the addition of excess of magnesium chloride to Ringer's solution on the response of the isolated guinea-pig uterus to pitressin.

Doses of Pitressin in ml.

A = Pitressin, 20 pressor units per ml.; dilution 1 : 120.
1·0 M = 1·0 mg. of magnesium chloride.

expressed as the percentage increase in the response, and these values are shown in column 3 of Table I. A number of observations were made upon different uteri for each concentration of excess magnesium chloride. The results of Table I are expressed graphically in Figure 2, dotted vertical lines indicating percentage standard error, and show that in a concentration of 0·00125 per cent. of magnesium chloride in excess of that in the normal Ringer's solution, the potentiation effect both to pitocin and pitressin is practically nil, whilst at the excess concentrations of 0·0025 per cent. and 0·005 per cent. of magnesium chloride the response to both pitocin and pitressin is greatly enhanced, the potentiation of pitressin being much greater than that of pitocin. From 0·01 per cent. to 0·05 per cent. increase is observed for both hormones, although there is no significant difference between the influence of excess of magnesium chloride in either case. At a concentration of 0·1 per

cent. of magnesium chloride in excess the potentiation of pitocin far exceeds that shown by pitressin.

In the main, the results support the work of Frazer[6], whereas his observations were made by introducing magnesium chloride into saline solution from which it was previously absent, the experiments described

TABLE I

THE INFLUENCE OF EXCESS OF MAGNESIUM CHLORIDE ON THE RESPONSE OF THE GUINEA-PIG UTERUS TO PITOCIN AND PITRESSIN

BASAL CONCENTRATION OF MAGNESIUM CHLORIDE IN RINGER SOLUTION =0·00025 per cent.

Excess Concentration of Magnesium Chloride	Number of Uteri	Increase in Response	
		Per cent.	Percentage Standard Error
		Pitocin	
0·00125	3	5	2·1
0·0025	4	43	4·4
0·005	2	82	2·2
0·01	6	70	26·6
0·025	4	45	24·8
0·05	8	46	24·8
0·1	2	187	3·0
		Pitressin	
0·00125	1	nil	
0·0025	2	129	9·0
0·005	3	104	10·6
0·01	2	44	10·0
0·025	3	59	18·1
0·05	4	95	30·5
0·1	2	76	2·0

above contained a minimum concentration of 0·00025 per cent. since uteri did not produce good contractions when stimulated with either pitocin or pitressin in a solution from which magnesium chloride was absent.

The second stage of the study of the effect of varying concentrations of magnesium ion comprised a series of assays of solutions with ratios of pitocin to pitressin from 4·0 down to 0·125 using the Ringer's solution proposed by (a) the British Pharmacopœia 1948 (magnesium chloride

TABLE II

TYPICAL DIFFERENCES IN GUINEA-PIG UTERINE ASSAY RESULTS USING TWO USUAL FORMULÆ FOR THE PHYSIOLOGICAL SALT SOLUTION

Ratio pitocin/pitressin	Actual total oxytocic potency U/ml. †	Ringer's solution B.P. 1948 potency found U/ml.	Ringer's solution with added Mg. (Hsu[5])*
4·00	10·1	8·0	10·6
2·00	10·2	9·2	10·6
1·00	10·4	10·0	10·6
0·50	10·8	9·3	13·6
0·25	11·6	11·2	13·6
0·125	13·2	13·0	19·2

* Hsu[5] Ringer contains 0·045 per cent. of magnesium chloride.

† Allowance has been made for 4 per cent. of oxytocic hormone impurity in pitressin and for 4 per cent. of pressor hormone impurity in pitocin in computing the actual total oxytocic potencies of the solutions.

concentration 0·00025 per cent.), and (b) Hsu[5] (magnesium chloride concentration 0·045 per cent.). The solutions were assayed against an extract of the International Posterior Pituitary Powder. The results are seen in Table II and show that the pressor principle exerts a significant oxytocic action when the ratio of pitocin to pitressin 0·50 or less,

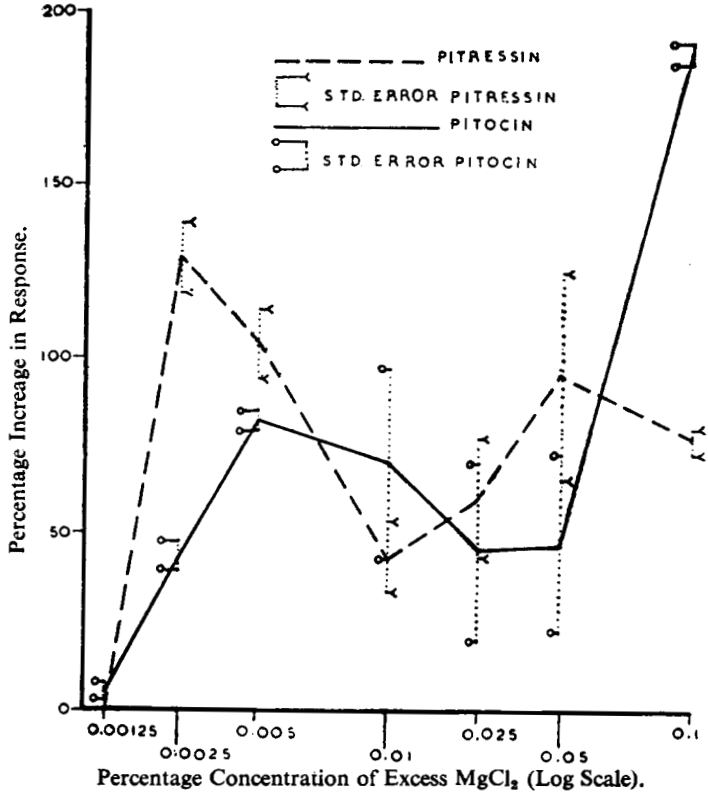

FIG. 2.—The effect of the addition of excess of magnesium chloride to Ringer's solution on the percentage increase in response of the isolated guinea-pig uterus to both pitocin and pitressin.

if Ringer's solution containing 0·045 per cent. of magnesium chloride is used. The potency found in Ringer's solution of the British Pharmacopœia 1948, for a mixture in which the ratio of pitocin to pitressin is 4·0 is significantly less than the actual potency, assuming a statistical limit of ±20 per cent.

VARIATION OF CALCIUM ION CONCENTRATION

Since previous workers[2,4], claimed that physiological salt solutions with concentrations of calcium chloride different from that which is used in normal Ringer's solution have led to the improvement of uterine responses to posterior pituitary extract, the effect of varied concentrations of calcium

chloride in the otherwise unchanged solution on the response of the uterus to pitocin and pitressin was studied next. The procedure followed was similar to that employed for the studies with excess of magnesium chloride. Figure 3 shows a section of a typical experiment in which the increase in the response to pitressin was studied after the addition of calcium chloride. Table III gives the results obtained, also expressed

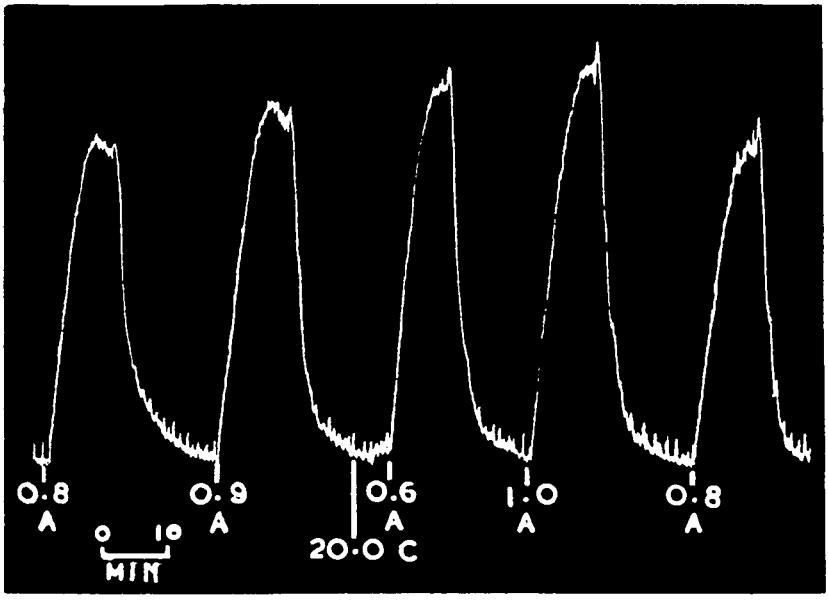

FIG. 3.—The influence of the addition of excess of calcium chloride to Ringer's solution on the response of the isolated guinea-pig uterus to pitressin.

Doses of Pitressin in ml.

A = Pitressin, 20 pressor units per ml.; dilution 1:120.

20·0 C = 20·0 mg. of calcium chloride.

graphically in Figure 4. At a concentration of 0·005 per cent. of calcium chloride in excess, pitocin shows a potentiation of 47 per cent. whilst there is no potentiation with pitressin; at concentrations of 0·01 per cent. and 0·025 per cent. in excess the potentiation with pitressin is greater than the potentiation with pitocin. There is no significant difference between the increase of pitocin and pitressin at a concentration of 0·05 per cent. of calcium chloride in excess, whilst at a concentration of 0·1 per cent. of the salt in excess it is the pitocin which shows the greater potentiation.

In the absence of calcium chloride from the Ringer's solution no response was given by the uterus to pitressin. The slight response that is shown in Figure 5 is probably due to a small amount of oxytocin as an impurity. Attempts to assay pitocin in the absence of calcium chloride were not successful. These observations on the effect of different concen-

TABLE III

THE INFLUENCE OF EXCESS OF CALCIUM CHLORIDE ON THE RESPONSE OF THE GUINEA-PIG UTERUS TO PITOCIN AND PITRESSIN

BASAL CONCENTRATION OF CALCIUM CHLORIDE IN RINGER'S SOLUTION =0·024 per cent.

Excess Concentration of Calcium Chloride	Number of Uteri	Increase in Response	
		Per cent.	Percentage Standard Error
		Pitocin	
0·0025	3	2	1·0
0·005	2	47	1·0
0·01	2	18	5·0
0·025	4	18	7·0
0·05	5	52	8·6
0·1	2	104	9·8
		Pitressin	
0·0025	7	—20	11·5
0·005	3	2	11·3
0·01	3	35	4·9
0·025	3	37	12·4
0·05	4	38	9·4
0·1	4	62	12·5

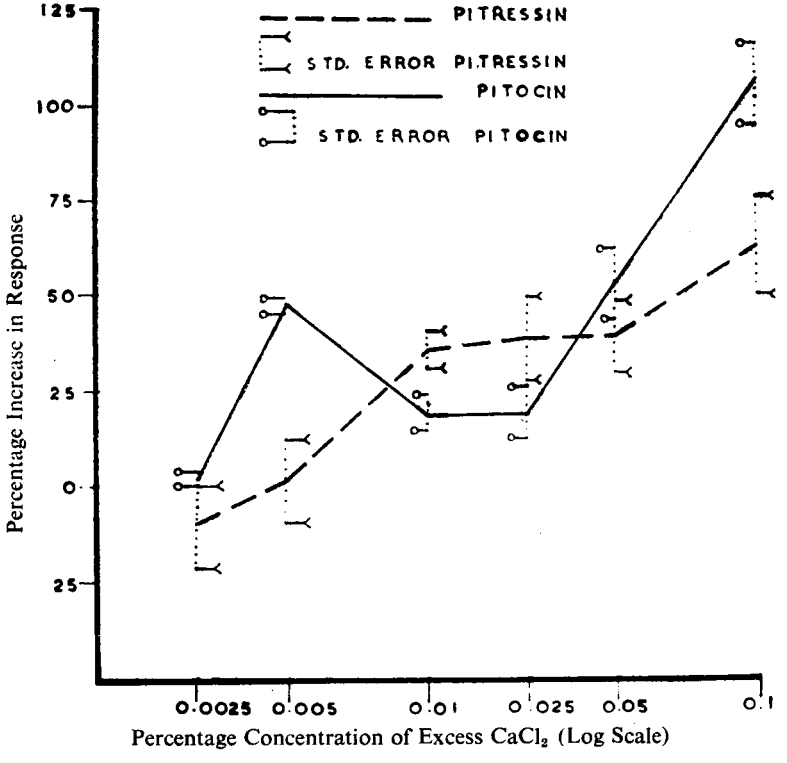

FIG. 4.—The effect of the addition of excess of calcium chloride to Ringer's solution on the percentage increase in response of the isolated guinea-pig uterus to both pitocin and pitressin.

trations of both magnesium chloride and calcium chloride indicate that unless the ratio of oxytocin to vasopressin is unity, that is, the ratio in which oxytocin and vasopressin occur in the International Standard Posterior Pituitary powder, then the true oxytocic potency of the test material

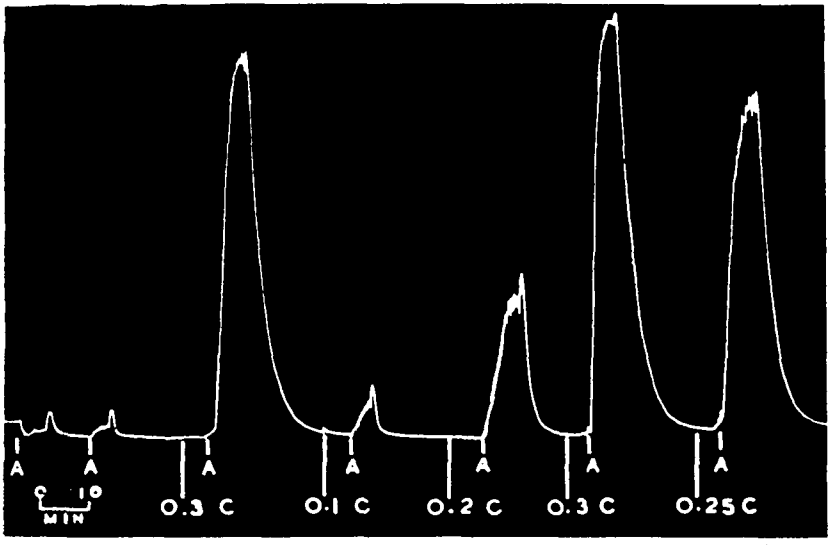

FIG. 5.—The influence of the addition of calcium chloride to Ringer's solution containing no calcium chloride on the response of the isolated guinea-pig uterus to pitressin.
A = 0·8 ml. of Pitressin, 20 pressor units per ml. dilution, 1 : 120.
C = Dose in mg. of calcium chloride.

cannot be calculated. Reference will be made later to work which has shown that this ratio can vary appreciably.

THE INFLUENCE OF DEXTROSE

Dextrose is a necessary constituent of the Ringer's solution, for in its absence the uterine muscle becomes rapidly fatigued by the first few doses of the posterior pituitary extracts, as Figure 6 indicates. In our experience dextrose at a concentration of that occurring in the normal Ringer's solution is sufficient to prevent the guinea-pig uterus from becoming fatigued for many hours.

THE EFFECT OF VARIABILITY OF THE UTERINE MUSCLE

Kochmann[2] and Morrell[8] and his co-workers have described the assay of posterior pituitary extracts using the uteri from virgin guinea-pigs in various stages of the sexual cycle, and the former even used uteri from pregnant guinea-pigs. The following experiments were undertaken to ascertain what differing effect, if any, pitocin and pitressin produced on the uteri of guinea-pigs treated with progesterone and stilbœstrol respectively. 1 mg./ml. of progesterone in arachis oil was administered subcutaneously in doses of 0·5 ml. per animal to a group of 6 virgin

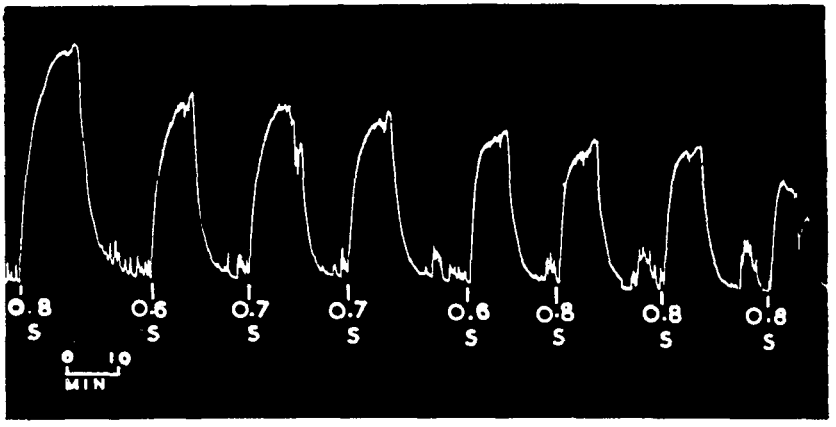

FIG. 6.—The response of the isolated guinea-pig uterus in Ringer's solution containing no dextrose to doses of pituitary extract.

S = ml. of Extract of International Posterior Pituitary Standard 2 I.U. per ml.; dilution 1:80.

albino guinea-pigs on each of 6 consecutive days. Stilbœstrol, 1 mg/ml. in arachis oil was given, at the same time, to another group of 6 virgin albino guinea-pigs each receiving 0·5 ml. subcutaneously on 6 occasions at intervals of 2 days. The concentration of drug at which the uterus just responded was taken as the limiting sensitivity of the uterus. The limits for pitocin and pitressin were observed, and the results are given in Table IV.

TABLE IV

THE SENSITIVITY TO PITOCIN AND PITRESSIN OF UTERI FROM VIRGIN GUINEA-PIGS TREATED WITH PROGESTERONE AND STILBŒSTROL

	Uterine Sensitivity		Sensitivity Ratio Pitocin/Pitressin
	Pitressin milliunits/ml	Pitocin milliunits/ml	
Progesterone-treated guinea-pigs	0·04 to 0·33	0·08 to 0·26	1·08
Stilbœstrol-treated guinea-pigs ...	0·01 to 0·04	0·002 to 0·018	2·50
Sensitivity ratio of progesterone-treated guinea-pigs to stilbœstrol-treated guinea-pigs	0·138	0·058	

The ratio of the sensitivity of pitocin and pitressin in the case of uteri from guinea-pigs treated with progesterone is 1·08, which indicates that pitressin exerts as great an oxytocic activity as pitocin, unit per unit, whilst in the case of the uteri from stilbœstrol-treated guinea-pigs the ratio is 2·50, pitocin thus exerting a greater oxytocic action than pitressin. The ratio of the sensitivity of the uteri from progesterone-treated guinea-pigs to those of stilbœstrol-treated ones, shows that in both cases the latter

treatment produces the greater sensitivity, and this is even more marked with pitocin than with pitressin. The actual tracings showed that the response to posterior pituitary extract with the uteri from stilbœstrol-treated guinea-pigs were extremely rapid, reaching their maximal contraction in a few minutes, and also that there was no base rhythm (see Fig. 7). In the case of the uteri from guinea-pigs treated with

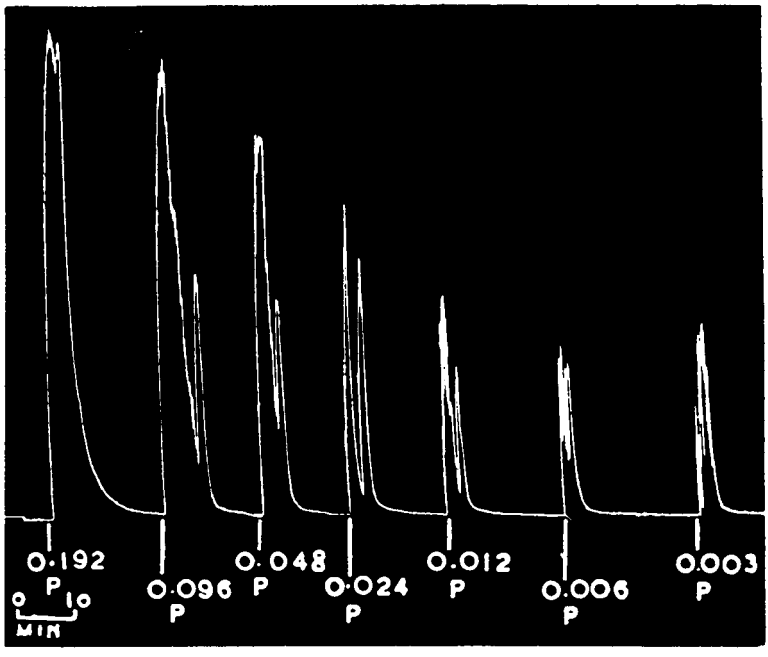

FIG. 7.—The response of the isolated uterus of a stilbœstrol-treated virgin guinea-pig to pitocin.

P = dose of pitocin in milliunits.

progesterone, however, the response to posterior pituitary extract was somewhat slower and the base rhythm was not abolished (see Fig. 8). Sufficient data were available to construct a histogram, shown in Figure 9, giving the average number of guinea-pigs, including failures, used for each assay every month during the period 1942 to 1946 inclusive. The blocks indicate the standard error. No one period of the year shows any very significant reduction in the number of guinea-pigs used per assay, though there is some indication that in July, August and September fewer guinea-pigs were used per assay than at other periods of the year, as indicated in the graph.

THE INFLUENCE OF TEMPERATURE

Hamburger[9] has reported on the influence of changes in temperature of the Ringer's solution upon the response of guinea-pig uteri to posterior

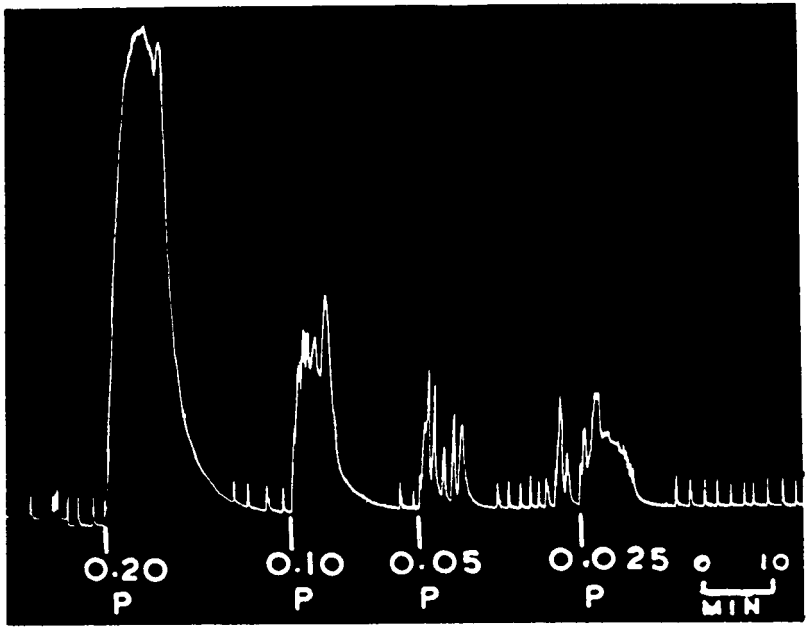

FIG. 8.—The response of the isolated uterus of a progesterone-treated virgin guinea-pig to pitocin.
P = dose of pitocin in milliunits.

pituitary extract. This effect we studied sufficiently to show that slight changes in temperature of the Ringer's solution do modify the response of the uterus to the hormonal extract, our observations being in close agreement with those of Hamburger. 37°C. is a suitable temperature at which to conduct the assay.

DISCUSSION OF UTERINE METHODS

Attempts have been made from time to time to establish an isolated uterine method of assay from which limits of error can be calculated. Hamburger[10] modified the method of Burn and Dale [3] and obtained for each guinea-pig uterus a number of contractions with posterior pituitary extract with doses ranging from that which produces maximum response to that producing minimum, and on the basis of the average height of the contractions for the individual doses plotted dose/response curves for the standard and unknown solutions. He introduced a dose into the bath every 10 minutes but in our experience this was not very practicable for rarely did the uterus relax before the introduction of the next dose into the bath. Introducing a dose immediately relaxation was achieved modified the subsequent response, but rhythm was not impaired.

Morrell[8] *et al.* published a method based on a quantal response, and used the uterus of the virgin guinea-pig weighing 280 to 480 g. Regression lines were computed from which the error of assay could be

calculated. They employed Van Dyke and Hastings'[7] solution which contains 0·005 per cent. of calcium chloride and 0·009 per cent. of magnesium chloride. A later paper published by Bachinski[11] *et al.* stated that although the method using the quantal response possessed several advantages over the official uterine method it lacked the precision obtainable with a graded type of response. This paper sets out a uterine

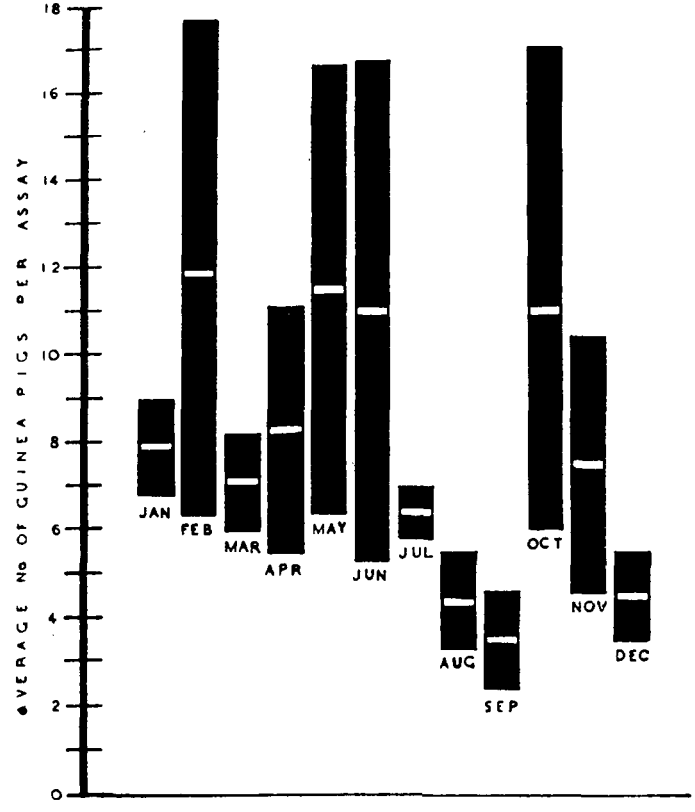

FIG. 9.—Histogram of the average number of virgin albino guinea-pigs used per assay for each month during the period 1942-46 inclusive. Narrow white strips indicate the mean values.

Blocks indicate the standard error.

method in which use is made of a quantitative response and yet the results stated in Table V of that paper show that 16 out of 23 results give percentage standard errors which do not include the true potency of the extracts assayed.

Holton[12] has recently described a precise and rapid method using the uterus of the rat maintained at 32°C. in a salt solution in which the calcium and glucose concentrations are respectively $\frac{1}{4}$ and $\frac{1}{2}$ that of Ringer's solution. Doses are randomised and limits of error are calculated. The mean percentage standard error is stated to be 2·16 from

8 satisfactory assays. The small limits of error have been confirmed in this laboratory though in one case they did not cover the true potency of a dilution of a commercial extract assayed against itself.

Of the four methods in use, three different modified Ringer's solutions are described, whilst the uteri from guinea-pigs in different stages of the sexual cycle have been used, and in one case the uterus from the rat.

Bachinski and Allmark[13] studied the oxytocic activity of pitocin and pitressin on isolated segments of uteri from the guinea-pig, rat, rabbit and man. The muscle strips were excised at the normal, parturient, and postpartum states. A marked difference in response to pitocin and pitressin was observed. The uteri of the normal rabbit more nearly approached that of the "*in vitro*" parturient human uterus in the relative oxytocic activity of pitocin and pitressin in a given pituitary extract. They used Van Dyke and Hastings'[7] solution since its magnesium chloride concentration approximates the serum level of the pregnant woman. Bachinski and Allmark[13] claim that any indirect method such as the chicken depressor method may not agree with an assay using rabbit or human uterine strips. The difficulty encountered with any isolated uterine method, however, is the inability to make the method workable in different laboratories under exactly similar conditions, and a criterion of a method of standardisation must be that samples assayed in one laboratory shall be found to have the same potency, within statistical limits of error, as the same samples assayed in other laboratories.

Of the uterine methods the rat uterine assay is the most accurate since statistical limits can be computed, but the rat uterus is not similar to the paturient human uterus, according to Bachinski and Allmark[13], and furthermore, in the light of the work which has been described in this paper there is reason to suspect that the rat uterus would be influenced by oxytocin and vasopressin to different degrees under variable conditions of assay. The factors involved are the variability of the different mammalian uteri to oxytocin and vasopressin, the sexual state of the uteri and the influence of one or all of the constituents of the physiological salt solutions in all their varying concentrations. Because of these factors the chicken depressor method for the assay of the oxytocic principle was examined.

The Chicken Depressor Method

Coon[14] gave a detailed account of the chicken depressor phenomenon and method of assay, and Thompson[15] later refined and modified the method to give a very precise assay from which limits of error could be calculated. Although Thompson gives one result which differs significantly from the value obtained by the guinea-pig uterine assay, the latter appears to have been conducted on one uterine strip only, and it has been our experience, in the performance of many hundreds of uterine assays, that occasionally an answer produced consistently by one uterus for a given sample of posterior pituitary extract cannot be produced with subsequent uteri. Because of this, uterine assays have always been performed on a number of uteri. As it was impossible to obtain white

Leghorn cockerels we used Rhode Island Red cockerels weighing 1·1 to 1·8 kg. The responses to pituitary extracts were very small indeed, and in two cases no response was produced at all. Light Sussex cockerels were in reasonable supply and these we finally adopted for the assay.

METHOD

The birds, weighing 1·8 to 2·3 kg. were anæsthetised by injecting phenobarbitone sodium 180 mg./kg. into the brachial vein. The popliteal artery and crural vein were dissected out and the blood pressure recorded from the former using a capillary mercury manometer. 8·5 per cent. of sodium citrate was used as anticoagulant. The International Standard Posterior Pituitary Extract, 2 oxytocic units per ml. was diluted 10 times with 0·9 per cent. saline solution and a volume not exceeding 0·4 ml. was injected rapidly into the crural vein. The doses were introduced into the vein at intervals of 3 to 5 minutes, but once the timing had been established it was strictly adhered to throughout the assay. A fall in blood pressure of 30 to 60 mm. of mercury was usually achieved. The unknown solution was diluted to a strength assumed to be equal to that of the Standard. Table V shows the results obtained in a comparison between the guinea-pig method of assay, using the Ringer's solution

TABLE V

COMPARISON OF THE GUINEA-PIG UTERUS AND CHICKEN BLOOD PRESSURE METHODS FOR THE MEASUREMENT OF OXYTOCIC ACTIVITY

Sample	Chicken depressor method U/g ± S.E. (C)	Guinea-pig uterus U/g. (G_p)	Ratio $\frac{C}{G_p}$
A	677 ± 52	700	0·967
B	872 ± 70	875	0·997
C	762 ± 49	637	1·196
D	792 ± 68	700	1·131
E	921 ± 49	900	1·023
F	785 ± 65	824	0·950
G	467 ± 44	500	0·945

proposed by the British Pharmacopœia 1948, and the chicken depressor method as described above. The 4th column, with the ratios C/G_p indicates the satisfactory agreement between the two methods of assay under the conditions stated.

Table VI gives the results obtained when mixtures of pitocin and pitressin were assayed. Pitressin exerted no oxytocic effect over a range of the ratio of pitocin to pitressin from 4·0 to 0·125. Coon found with white Leghorns that the ratio of oxytocin to vasopressin should not be less than 0·4 otherwise the vasopressin would influence the oxytocic response. The chicken depressor method has the added advantage over the guinea-pig uterine method is, that it is easy to perform and the pressor principle does not interfere with the ratio of pitocin to pitressin of 0·125. As many as 9 samples have been assayed on one bird in a day with 8 to 10 doses of the unknown per assay. As with the uterine assay, the results obtained with any one animal, however, should not be used unless confirmed at least once again on another bird.

As the guinea-pig uterine method is still the official method for assaying posterior pituitary extracts, this test is also performed in these laboratories, and confirmation has been obtained in 71 cases between

TABLE VI

THE INFLUENCE OF DIFFERENT RATIOS OF PITOCIN TO PITRESSIN ON THE ASSAY OF THE OXYTOCIC PRINCIPLE OF THE POSTERIOR PITUITARY LOBE GLAND BY THE CHICKEN DEPRESSOR METHOD

Ratio pitocin/pitressin	Actual total oxytocic potency U/ml*	Potency found	Limits of error (P = 0·95)
4·00	10·1	10·3	9·3 to 11·3
2·00	10·2	11·2	8·9 to 13·5
1·00	10·4	9·7	8·4 to 11·0
0·50	10·8	10·8	10·0 to 11·6
0·25	11·6	11·1	10·1 to 12·1
0·125	13·2	11·1	8·3 to 13·9

* Allowance has been made for 4 per cent. of oxytocic hormone impurity in pitressin and for 4 per cent. of pressor hormone impurity in pitocin in computing the actual total oxytocic potencies of the solutions.

this method and the chicken depressor method. Furthermore, the chicken assay is a less costly test than the official method, and its greatest advantage lies in the fact that limits of error can be calculated and are of small magnitude.

THE STUDY OF COMMERCIAL POSTERIOR PITUITARY POWDERS AND EXTRACTS

Recent investigations into the large-scale extraction of posterior pituitary lobe powders revealed the fact that the oxytocic and pressor ratios in the extracts are not necessarily unity. In Table VII the results

TABLE VII

EXTRACTION OF COMMERCIAL SAMPLES OF POSTERIOR PITUITARY LOBE POWDER UNDER DIFFERENT CONDITIONS OF MANUFACTURE

Treatment	Pressor activity U/g.	Oxytocic (depressor) activity U/g. ± Std error U/g.	Ratio oxytocin/vasopressin
A	888	883 ± 56	1·00
B	552	677 ± 52	1·22
C	482	196 ± 14	0·41
D	658	508 ± 21	0·77

of four different extraction treatments are recorded. The ratio of oxytocin to vasopressin for treatment C is 0·41 and is significantly greater than that of any other. McClosky[16] *et al.* has shown that in whale hypophysis also the ratio of vasopressin to oxytocin is greater than unity.

The method adopted for the assay of pressor activity was described by Kamm[17] and his co-workers. Table VIII shows the results obtained in this laboratory for commercial extracts when assayed using the Ringer's solution proposed by the British Pharmacopœia. All the products

were labelled 10 oxytocic units/ml. and their potencies range from 7·7 to 11·0 oxytocic units/ml.

Figure 10 is a tracing of an actual test, which is performed by obtaining two different submaximal responses to the extract of posterior pituitary standard. Next a dose of the unknown which gives a response intermediate between those of the two different standard responses is found. The three responses are repeated in a reverse sequence. Such a method shows whether or not rhythm has been introduced, in the latter

TABLE VIII

RESULTS OBTAINED FOR THE OXYTOCIC ACTIVITY OF COMMERCIAL POSTERIOR PITUITARY EXTRACTS, LABELLED TO CONFORM WITH THE B.P. REGULATIONS OF 10·0 OXYTOCIC UNITS PER ML., WHEN ASSAYED BY THE GUINEA-PIG UTERUS METHOD USING RINGER'S SOLUTION B.P. 1948

Sample	Potency U/ml	Limits U/ml*
A	10·0	8·6 to 11·4
B	8·8	7·5 to 10·0
C	7·8	6·7 to 8·9
D	11·0	10·0 to 12·2
E	8·8	7·5 to 10·0
F	7·8	6·7 to 8·9

* Range of upper and lower doses used in bracketting arrangement described for the uterine assay.

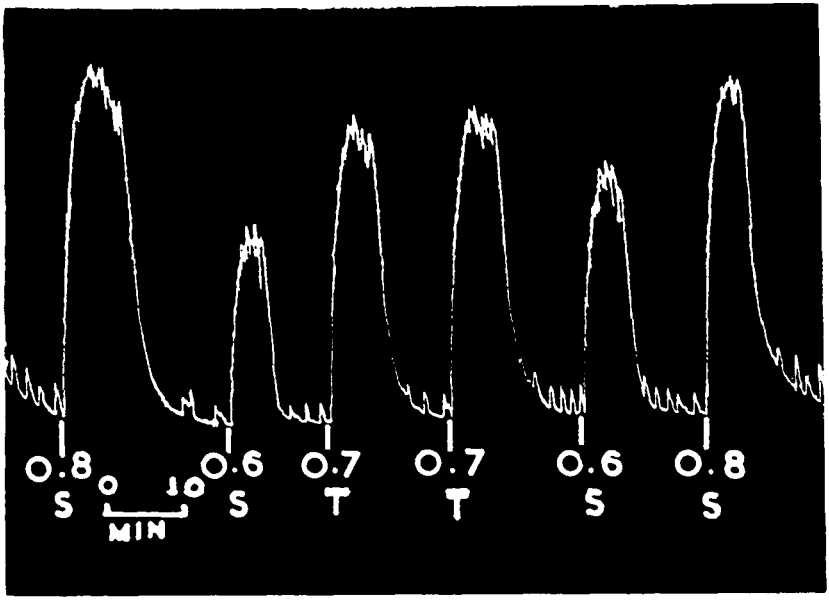

FIG. 10.—Typical example of the isolated guinea-pig uterine assay of a posterior pituitary extract.

S=ml. of Extract of the International Posterior Pituitary Standard 2 I.U. per ml.; dilution 1:120.

T = ml. of Unknown; dilution 1:600.

case the test is discarded. At least two such complete tests are obtained from different uteri to constitute an assay.

SUMMARY

Work has been described which shows the errors which may be encountered under different experimental conditions of the uterine assay of oxytocin, or commercial posterior pituitary extracts where the ratio of oxytocin to vasopressin is not unity.

1. By increasing the concentration of magnesium chloride in the British Pharmacopœia 1948 Ringer's solution, the response of the guinea-pig uterus to pitocin is increased.

2. The response of the guinea-pig uterus to pitressin is also potentiated by increasing the concentration of magnesium chloride, but to a different extent than is pitocin.

3. Different concentrations of calcium chloride in the Ringer's solution produce potentiation to both pitocin and pitressin, but to different extents.

4. The pressor principle does not produce any oxytocic effect on the uterus suspended in Ringer's solution from which calcium chloride is absent.

5. The absence of glucose from the Ringer's solution produces rapid fatiguing of the uterine strip.

6. Temperature regulation of the Ringer's solution is very important.

7. The sensitivity of uteri from guinea-pigs treated with stilbœstrol is greater than the uteri of progesterone-treated guinea-pigs to both pitocin and pitressin.

8. The ratio of the sensitivity of uteri from progesterone-treated guinea-pigs to pitressin and pitocin is almost unity, whilst the ratio of sensitivity for the uteri of guinea-pigs treated with stilbœstrol indicates that the uteri are more sensitive to pitocin than to pitressin.

9. Seasonal variation in the number of virgin albino guinea-pigs used per assay was not found to be significant.

10. The ratio of oxytocin to vasopressin in the test samples must be the same as the ratio which is stated for the standard.

11. The type of uterus and the physiological salt solution should be stated.

12. It is recommended that the modification of Ringer's solution proposed by the British Pharmacopœia 1948 should be used for the guinea-pig assay, and that the uterus of the guinea-pig should be that of a virgin in dioestrus.

13. The chicken blood depressor method offers many advantages over the isolated guinea-pig uterine method. It is cheaper and quicker to perform, and is more accurate in that narrow fiducial limits can be obtained.

14. Until the chicken blood depressor method has been studied exhaustively in many laboratories engaged on the assay of the oxytocic

principle, the guinea-pig uterine assay should be run in parallel with the former.

I wish to thank Dr. R. H. Thorp for his guidance and helpful criticisms, Miss B. Bridgland for technical assistance and the Directors of the Wellcome Foundation, Ltd., for permission to publish this paper.

REFERENCES

1. Dale and Laidlaw, *J. Pharmacol.*, 1912, **4,** 75.
2. Kochman, *Hoppe-Seyl. Z.*, 1921, **115,** 305.
3. Burn and Dale, *Spec. Rep. Ser. Med. Res. Coun., Lond.*, 1922, No. 69.
4. de Jalon, *Farmacoter. Act.*, 1947, **4,** 177.
5. Hsu, *Quart. J. Pharm. Pharmacol.*, 1948, **21,** 146.
6. Frazer, *J. Pharmacol.*, 1939, **66,** 85.
7. Van Dyke and Hastings, *Amer. J. Physiol.*, 1928, **83,** 563.
8. Morrell, Allmark and Bachinski, *J. Pharmacol.*, 1940, **70,** 440.
9. Hamburger, *Acta Pharmacol. Toxicol.*, 1946, **2,** 212.
10. Hamburger, *Acta Pharmacol. Toxicol.*, 1945, **1,** 112.
11. Bachinski, Allmark and Morrell, *Canad. J. Res.*, 1945, **E23,** 126.
12. Holton, *Brit. J. Pharmacol.*, 1948, **3,** 328.
13. Bachinski and Allmark, *J. Amer. pharm. Ass., Sci. Ed.*, 1947, **36,** 73.
14. Coon, *Arch. int. Pharmacodyn.*, 1939, **62,** 79.
15. Thompson, *J. Pharmacol.*, 1944, **80,** 373.
16. McClosky, Miller and Le Messurier, *J. Pharmacol.*, 1936, **57,** 132.
17. Kamm, Aldrich, Grote, Rowe and Bugbee, *J. Amer. chem. Soc.*, 1928, **50,** 573.