assay of pituitary gonadotrophin content in starved rats. They pointed out that other investigators implanted entire pituitary glands into recipient assay rats, whereas they injected suspensions of pituitary tissue, as was also done in our experiments. The implantation method is believed to be less exact since necrosis, growth, or encapsulation may occur, resulting in an exceedingly variable delivery of gonadotrophic hormone.

It is not clear as to why the pituitary gonadotrophin content remains the same during periods of starvation while pituitary lactogen becomes reduced. There can be no doubt that starvation, depending on its degree, results either in a reduction or complete cessation of gonadal and mammary gland activity. This would certainly seem to indicate that the amounts of circulating gonadotrophic and lactogenic hormones are reduced.

On the assumption that pituitary content of a hormone represents the resultant between the amount being produced and the amount released into the blood stream, Maddock and Heller[10] explain the 'dichotomy' between gonadotrophin content in the pituitary and blood stream of starved rats as being due to a failure of release and to a minimum of production by the pituitary. The decrease of lactogenic hormone content in the pituitary during starvation could be explained as being due primarily to a failure of production with little or no interference with the release mechanism.

Assays of pituitary content of gonadotrophin have proven to be reliable indicators of the amounts present in the circulation following ovariectomy, during estrogen administration, but not during starvation. Assays of the lactogen content of the pituitary have accurately reflected the amounts present in the blood stream during lactation, during estrogen or testosterone administration, and also during periods of restricted feed intake.

*Summary.* The lactogenic and gonadotrophic hormone content of pituitaries of intact and ovariectomized rats was determined after full, ¾, ½, ¼ and no-feed regimes. The full or partially-fed groups were sacrificed at the end of 14 days and the unfed group at the end of 7 days. Pituitary lactogen content was reduced in the intact and ovariectomized rats on the ½, ¼ and no-feed regimes below that of the ¾ and full-fed controls. No change occurred in the gonadotrophin content of the pituitaries of either the intact or ovariectomized rats, regardless of the level of feed intake. In the intact but not in the ovariectomized rats, restricted feed intake caused a marked reduction in the weights of the pituitary, thyroid and adrenals, except that on the no-feed regime adrenal weight was increased in both groups.

## 16977
## Assay of Secretin and Cholecystokinin Concentrates.*

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The most feasible assay of secretin concentrates involves the cannulation of the pancreatic duct and observation of the increased rate of pancreatic juice output upon intravenous injection of the sample. Ivy, Kloster, Drewyer and Lueth[1] introduced the threshold dose, namely, that amount of a secretin concentrate causing a flow of 10 drops (0.4 ml) of pancreatic juice within a 10-minute period over the control rate in the dog. In a later work, Greengard and Ivy[2] reported that the threshold dose of a preparation obtained from hog duodenums (SI) and adopted as a standard was 0.25 mg for many dogs. This

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1 Ivy, A. C., Kloster, G., Drewyer, G. E., and Lueth, H. C., *Am. J. Physiol.*, 1930, **95**, 37.

2 Greengard, H., and Ivy, A. C., *Am. J. Physiol.*, 1938, **124**, 427.

value was employed for assay in conjunction with the ratio of weights of unknown to standard eliciting a 10 drop response in 10 minutes. Still[3] determined secretin unitage from the ratio of drops of unknown to a vasodilatin-free standard produced by the injection of 4 mg of each. Neither sample data nor comments as to reproducibility were given. Penau and Simonnet[4] derived a unit from uniform injections of secretin concentrates and measurement of juice output after 15, 30 and 45 minutes. A high variability resulted. A marked departure from the above direct methods is that of Wilander and Ågren[5] who employed narcotized cats. A unit was arrived at from the titration of the alkalinity of secretions from the duodenum soaked up by a rolled-up filter paper. Among others, a criticism of this method is that the alkalinity is not due to pancreatic juice alone. A comparison of this cat unit with the one obtained by Greengard and Ivy has been made.[2,6]

A direct method for the assay of cholecystokinin (CCK) fractions described by Ivy and Oldberg[7] involves cannulation of the gall bladder after clamping the cystic duct. Contractions were observed by connection to a recording tambour. A unit of CCK potency was defined as that amount of vasodilatin-free concentrate which when injected intravenously brings about a gall bladder contraction of one centimeter of bile. Recently[8] a method has been reported for CCK estimation which involves the recording of an increase in the pressure in the common bile duct after the injection of CCK in unanesthetized dogs, the common bile duct being cannulated through a duodenal fistula. The *in vitro* contraction of guinea pig[9] and frog[10] gall bladders has been used for assaying CCK preparations. However, numerous difficulties are encountered with this method, notably, the regulation of intravesical pressures. In the present report, details are given for the assay of secretin and CCK, both of which may be carried out on the same dog. New reproducible units of potency are derived for these hormones by comparison of the gall bladder or pancreatic response produced by the standard with that of a test concentrate.

*Preparation of the Standard Concentrate.* A representative batch of SI prepared by treatment of a hog duodenal extract with trichloroacetic acid according to the method of Greengard and Ivy[2] was adopted as the standard. Although a few products proved to be somewhat more potent, the standard concentrate was chosen on the basis of the absence of vasodilatins. The dry standard has been stored at room temperature for over 2 years with no detectable loss in potency. A freshly prepared aqueous or dilute hydrochloric acid solution is used in the assay.

*Assay Procedure.* Dogs, 9-13 kg in weight, which have been fasted for at least 19 hours are placed under pentobarbital anesthesia. The main pancreatic duct is cannulated and after filling of the cannula, a rubber or plastic tube containing saline is connected so as to register juice output on a drop recorder. Carotid blood pressure is recorded throughout. A priming dose of secretin concentrate is injected into the exposed femoral vein, and, after some time, the basal rate of flow is determined. This is useful in the exploration of a practical drop rate especially where a pancreas is hypersecretive. A known amount of standard SI is injected and the number of drops of pancreatic juice, which decreases rapidly in rate with time, is recorded over a 10-minute period. After basal conditions prevail, the test concentrate is then administered in an amount yielding the same drop count within one to two drops over the same time interval. The ratio of standard to test sample dosages is

[3] Still, E. U., *Physiol. Rev.*, 1931, **11**, 342.

[4] Penau, H., and Simonnet, H., *Cong. Pharm.* (Liège, 1934), **1935**, 145; *C. A.*, 1936, **30**, 4893.

[5] Wilander, O., and Agren, G., *Biochem. Z.*, 1932, **250**, 489.

[6] Greengard, H., and Stein, I. F., Jr., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 149.

[7] Ivy, A. C., and Oldberg, E., *Am. J. Physiol.*, 1928, **86**, 599.

[8] Snape, W. J., Friedman, M. H. F., and Thomas, J. E., *Gastroenterology*, 1948, **10**, 496.

[9] Jung, F. T., and Greengard, H., *Am. J. Physiol.*, 1933, **103**, 275; Doubilet, H., and Ivy, A. C., *Am. J. Physiol.*, 1938, **124**, 379; Ågren, G., *Skand. Arch. Physiol.*, 1934, **81**, 234.

[10] Seager, L. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 326; 1941, **47**, 257.

determined. Further samples may be assayed with frequent checking of the standard dosage.

For the assay of cholecystokinin, the cystic duct is clamped or ligated after isolating it from the cystic artery, and a grooved metal cannula is secured in the gall bladder. A glass tube of 8 mm outside diameter is connected between the cannula and the recording tambour. Where an insufficient amount of fluid is present in the glass tube, additional bile is added. The standard SI is injected and the gall bladder contraction, which is complete within the first few minutes, is recorded in millimeters by noting the initial and final bile levels in the glass tube. When virtually the same initial level is restored, the test extract is introduced in such dosage that the same rise is obtained within 1 ml; however, for a rise of 10 mm or more, 2 mm are often allowed in the comparison, depending on the sensitivity of the gall bladder. The ratio of standard to test dosages is then determined. Since the response to the standard often varies with time and the condition of the dog, frequent or alternate checking is necessary.

It is important in the comparison of a test sample with the standard to treat both in a similar manner especially as regards the initial bile-pressure level, and readings must be taken with consistency particularly in the case of hypersensitive gall gladder preparations. For assay purposes, it was found that more accurate results are obtained by measuring the liquid rise in the glass tube than by comparing the respective heights on the tambour tracing. Secretin and CCK assay values can be checked by employing a comparison at a different drop rate or gall bladder contraction, respectively. The use of derived standards or concentrates of known potency repeatedly checked against the SI standard is also of value.

Since the presence of vasodilatins in a concentrate can have a marked effect on the secretin and CCK assay results, the blood pressure recording must be closely followed. Where additional pentobarbital is administered, sufficient time should elapse before the animal is restandardized.

*Variation of Gall Bladder Response with CCK Dosage.* A study of the response of the gall bladder to varying doses of CCK concentrates was carried out on several dogs. For some of the large contractions, time intervals up to 45 minutes were required before initial conditions were restored. The majority of the data was rechecked.

*Results.* Sample data typical for the secretin assay are shown in Table I for 6 preparations carried out on a number of dogs. The respective ratios did not change on comparison of the standard and the test concentrate at a different common drop rate. The value of a respective potency ratio is taken as the number of units of secretin contained in 1 mg of the concentrate. Table II illustrates the potency ratios or CCK unitage per mg for 5 fractions with good reproducibility in almost all cases. The concentration of the standard SI solution was usually 1 mg per ml. Although more potent concentrates were diluted so that the injected volumes of test and standard solutions were similar in many cases, no significant deviations resulted when they differed.

*CCK Dose-Response Relationships.* By plotting the respective responses or gall bladder contractions against gradually increasing doses of CCK, curves were obtained of the type given in Fig. 1. Except for curve III obtained with a concentrate of 2.0 units of CCK per mg, standard SI was employed.

*Discussion.* The results of the assay methods outlined for secretin and CCK show clearly that reproducible ratios are obtained which are independent of the weight of the test animal. Furthermore, where the condition of the dog may change after the determination of a ratio, restandardization often gives the same value. This discovery renders the determination of an Ivy secretin threshold dose less reliable than the method herein described. As can be seen from the fluctuation of dosage for a given drop rate from one dog to another in Table I, the former method of assay required at least 10 dogs for an acceptable assay.

The values of the potency ratios are equated to unitage of secretin or CCK contained in 1 mg of the concentrate. The results could be equally well expressed in multiples of these

TABLE I.
Assay Values for Secretin Preparations.

| | Dog No. | Preparation, mg | SI standard, mg | Pancreatic juice, drops | Secretin potency ratio (units secretin/mg) |
|---|---|---|---|---|---|
| Prep. S-61 | 16 | 1.0 | 2.5 | 12 | 2.5 |
| | 17 | 1.3 | 3.0 | 9 | 2.3 |
| | 51 | .81 | 2.0 | 28 | 2.5 |
| | 57A | .46 | 1.1 | 11 | 2.4 |
| | 58 | .75 | 1.8 | 38 | 2.4 |
| | 59 | 1.3 | 3.2 | 37 | 2.5 |
| | 67 | 1.25 | 3.0 | 11 | 2.4 |
| Prep. S-14 | 1 | 2.0 | 3.0 | 10 | 1.5 |
| | 16 | 1.5 | 2.5 | 9 | 1.7 |
| | 17 | 2.7 | 4.0 | 10 | 1.5 |
| | 25 | 3.1 | 4.5 | 14 | 1.5 |
| | 47 | 1.95 | 2.9 | 10 | 1.5 |
| | 48 | 2.8 | 4.2 | 25 | 1.5 |
| | 49 | 1.25 | 2.0 | 14 | 1.6 |
| | 51 | 1.3 | 2.0 | 15 | 1.5 |
| | 58 | 1.2 | 1.8 | 27 | 1.5 |
| | 61 | 4.0 | 6.0 | 11 | 1.5 |
| Prep. S-75 | 1 | 1.5 | 3.0 | 10 | 2.0 |
| | 12 | 5.0 | 10.5 | 9 | 2.1 |
| | 47 | 1.3 | 2.6 | 14 | 2.0 |
| | 51 | 1.0 | 2.0 | 25 | 2.0 |
| | 54 | .60 | 1.2 | 9 | 2.0 |
| | 55 | 1.2 | 2.4 | 10 | 2.0 |
| | 56 | .50 | 1.05 | 40 | 2.1 |
| | 57A | .55 | 1.1 | 16 | 2.0 |
| | 58 | .90 | 1.8 | 27 | 2.0 |
| | 59 | 1.6 | 3.2 | 50 | 2.0 |
| Prep. S-73 | 12 | 2.0 | 10.5 | 10 | 5.3 |
| | 25 | .80 | 4.5 | 19 | 5.6 |
| | 26 | .95 | 5.0 | 10 | 5.3 |
| | 40 | .075 | 0.40 | 14 | 5.3 |
| | 41 | .28 | 1.5 | 17 | 5.4 |
| | 47 | .50 | 2.7 | 8 | 5.4 |
| | 51 | .38 | 2.0 | 12 | 5.3 |
| | 59 | .60 | 3.2 | 40 | 5.3 |
| | 64 | .56 | 3.0 | 40 | 5.4 |
| | 65 | .40 | 2.2 | 30 | 5.5 |
| | 67 | .50 | 2.8 | 21 | 5.6 |
| Prep. S-165 | 26 | .39 | 5.0 | 12 | 13 |
| | 36 | .32 | 4.0 | 9 | 12.5 |
| | 41 | .080 | 1.0 | 9 | 12.5 |
| | 53 | .12 | 1.5 | 13 | 12.5 |
| | 58 | .14 | 1.8 | 27 | 13 |
| | 68 | .16 | 2.0 | 28 | 12.5 |
| | 71 | .20 | 2.5 | 33 | 12.5 |
| | 72 | .23 | 3.0 | 10 | 13 |
| | 73 | .25 | 3.0 | 17 | 12 |
| | 74 | .40 | 4.8 | 27 | 12 |
| Prep. S-36 | 66 | .25 | 7.6 | 15 | 30 |
| | 67 | .10 | 3.0 | 11 | 30 |
| | 70 | .12 | 3.6 | 20 | 30 |
| | 72 | .10 | 3.0 | 10 | 30 |
| | 73 | .10 | 3.0 | 19 | 30 |
| | 76 | .10 | 3.0 | 32 | 30 |
| | 77 | .10 | 3.0 | 20 | 30 |
| | 83 | .075 | 2.2 | 12 | 29 |

TABLE II.
Assay Values for CCK Concentrates Compared Against Standard SI.

| | Dog No. | Preparation, mg | SI standard mg | Gall bladder contraction, mm | CCK potency ratio (units CCK/mg) |
|---|---|---|---|---|---|
| Prep. C-4 | 16 | 4.0 | 6.5 | 10 | 1.6 |
| | 25 | 3.1 | 4.5 | 11 | 1.5 |
| | 40 | .50 | .75 | 12 | 1.5 |
| | 47 | 1.9 | 2.4 | 8 | 1.3 |
| | 48 | 2.7 | 4.2 | 7 | 1.6 |
| | 49 | 1.25 | 2.0 | 15 | 1.6 |
| | 50 | 1.25 | 2.0 | 8 | 1.6 |
| | 51 | 1.3 | 2.0 | 10 | 1.5 |
| | 58 | 1.2 | 1.8 | 23 | 1.5 |
| | 61 | 4.0 | 6.0 | 6 | 1.5 |
| Prep. C-7 | 9 | 4.5 | 9.0 | 9 | 2.0 |
| | 37 | 8.0 | 16.5 | 9 | 2.1 |
| | 47 | 1.3 | 2.6 | 9 | 2.0 |
| | 49 | .40 | .80 | 8 | 2.0 |
| | 51 | 1.0 | 2.0 | 10 | 2.0 |
| | 54 | .60 | 1.2 | 10 | 2.0 |
| | 55 | 1.2 | 2.4 | 10 | 2.0 |
| | 56 | .50 | 1.05 | 8 | 2.1 |
| | 57A | .55 | 1.1 | 5 | 2.0 |
| | 58 | .90 | 1.8 | 20 | 2.0 |
| Prep. C-60 | 15 | 3.5 | 9.0 | 10 | 2.6 |
| | 50 | .84 | 2.3 | 5 | 2.7 |
| | 51 | .75 | 2.0 | 10 | 2.7 |
| | 56 | .39 | 1.0 | 7 | 2.6 |
| | 58 | .73 | 1.8 | 24 | 2.5 |
| | 67 | 1.25 | 3.0 | 20 | 2.4 |
| Prep. C-37 | 12 | 1.2 | 5.0 | 10 | 4.2 |
| | 16 | 1.5 | 6.5 | 10 | 4.3 |
| | 25 | .90 | 4.5 | 11 | 5.0 |
| | 40 | .090 | .40 | 10 | 4.4 |
| | 47 | .50 | 2.2 | 7 | 4.4 |
| | 51 | .46 | 2.0 | 9 | 4.3 |
| | 64 | .70 | 3.0 | 11 | 4.3 |
| | 67 | .70 | 3.0 | 17 | 4.3 |
| | 68 | .70 | 3.0 | 19 | 4.3 |
| Prep. C-100P | 152 | .30 | 3.5 | 11 | 12 |
| | 161 | .46 | 5.5 | 10 | 12 |
| | 170 | .60 | 7.5 | 35 | 12.5 |
| | 175 | .20 | 2.5 | 22 | 12.5 |

values or in terms of any arbitrary unitage or percentage. Although comparison of test fractions can also be affected by reference to more potent standards, SI has been chosen on the basis of its uniformity of preparation and the presence of both secretin and CCK.

Any method for the biological assay of secretin is fraught with difficulties. For unknown causes, some dogs are very insensitive to secretin. In some cases, after a small dose of secretin is injected the increase in the flow of juice is sustained for longer than 10 minutes. Also, in the case of the CCK assay, it must be remembered that a smooth muscle response is involved. Aside from difficulties arising from the inability of separating the cystic artery and trauma to the gall bladder, some preparations are quite insensitive and, in some cases, the initial conditions of intragall bladder pressure are not attained. Only well-functioning gall bladder preparations should be employed for the assay of concentrates. A large number of these difficulties are minimized by employing the present method of comparing the response of test concentrate with that of the standard.

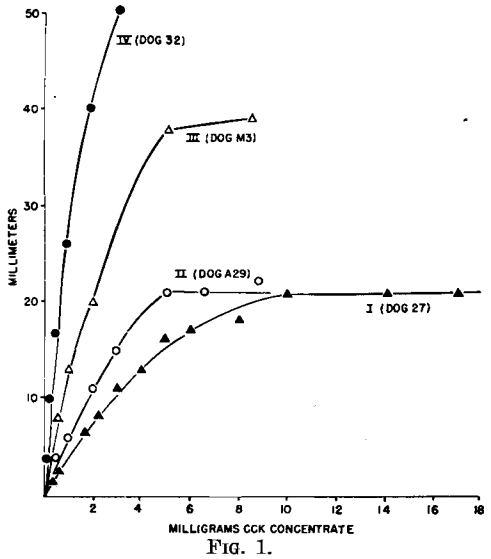


FIG. 1.

Response of the gall bladder to varying doses of cholecystokinin. Curves I, II and IV represent Standard SI; III was obtained for Preparation C-7 containing 2.0 units CCK/mg.

As might be predicted, the administration of increasing doses of CCK brings about gall bladder contractions which increase until a maximum is attained, and beyond which there is no further increase in response in spite of further doses of CCK. This maximum contraction which corresponds to complete emptying of the viscus, varies from one dog to another and is definitely affected by the volume of the gall bladder. Actually, in the CCK assay, volume changes are involved and well-filled gall bladders are preferable. In the comparison of the test sample with the standard, a contraction lower than the maximum must be employed, and this can be checked by noting if a greater bile-pressure rise occurs upon increasing this dosage. Large contractions are avoided, since often a longer time is required for the attainment of initial conditions.

The dose response curve for secretin has been shown by Greengard, Stein and Ivy[11] to be S-shaped in character, and Burn and Holton[12] have used the lower linear portions for the comparison of secretin concentrates with their standard which is comprised of an acetone-dried duodenal powder. Since a maximum in drop response is obtained with elevated doses of secretin, comparisons must be made at drop counts below this limiting value. It should also be pointed out that the log dose-response curves, which are linear for CCK and secretin in the region far removed from their respective maxima, could also be applied to the assay of concentrates. However, it is relatively simple to determine the requisite potency ratio in subsequent dogs by the method outlined in this paper after a first value has been obtained. Usually 3 dogs are sufficient as can be noted in Tables I and II.

*Summary.* The assay of secretin and CCK concentrates are affected by cannulation of the pancreatic duct and the gall bladder, respectively. The ratio of dosages of a vasodilatin-free standard concentrate (SI) to the test fraction yielding the same number of drops of pancreatic juice in a 10-minute interval is determined, and the value is taken as the secretin unitage contained in 1 mg of test fraction. Similarly, the dosage ratio of this standard to the unknown giving the same gall bladder contraction is equated to the units of CCK per mg of unknown. Good reproducibility is obtained in a large number of dogs.

The dose-response curves for CCK and their significance are discussed.

The authors are grateful to Dr. R. W. Denton for aid in surgical techniques, and to Mr. Max Krup for independent checking of a number of the assays.

11 Greengard, H., Stein, I. F., Jr., and Ivy, A. C., *Am. J. Physiol.*, 1941, **132**, 305.

12 Burn, J. H., and Holton, P., *J. Physiol.*, 1948, **107**, 449.