seems nearly infinitesimal when compared with the magnitude of the volume of the glomerular filtrate, it would seem important nevertheless from the point of view of chloride balance. Physiologically and pathologically, this appears significant, especially since, in the nephrotic syndrome, the excretion of sodium chloride is impaired, and its retention, along with water, gives rise to the pathologic accumulation of edema fluid. These considerations gain added importance, since Binger, Keith, and one of us (Goudsmit)[1] showed comparable increases of the rates of excretion of chloride in patients having nephrotic edema under treatment with solution of acacia.

Plasma Proteins. In all experiments, the concentration of plasma proteins was decreased, following the injection of a solution of acacia. This decrease was roughly proportional to the dose of acacia, and appears to have been caused chiefly by dilution, since the change in the concentration of plasma proteins can be rather satisfactorily predicted from the changes in the values of the hematocrit. The deviation of the average predicted change in plasma proteins from the average change observed is 3%. The concentrations of acacia in the plasma observed subsequent to the completion of its injection varied between 1210 and 3100 mg per 100 cc.

Summary. In experiments on dogs, after the intravenous injection of acacia, it was found that (1) the rate of glomerular filtration is essentially unchanged; (2) water excretion shows a diphasic response; (3) chloride excretion is markedly increased; (4) plasma proteins and hematocrit values diminish in comparable extent.

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Further Studies on a Frog Method for Assaying Gallbladder-Contracting-Substances.

LLOYD D. SEAGER.

From the Departments of Pharmacology, St. Louis University and University of Tennessee, Memphis, Tenn.

Cholecystokinin is usually assayed by the dog method,[1] incompletely described, and by the guinea pig method.[2, 3] Both are time

[1] Goudsmit, Arnoldus, Jr., Binger, M. W., and Keith, N. M., unpublished data.

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

[2] Agren, Gummar, *Skand. Arch. J. Physologie*, 1934, **69**, 1.

[3] Doubilet, H., and Ivy, A. C., *Am. J. Physiol.*, 1938, **124**, 379.

consuming and expensive in animal materials. In a previous communication,[4] I have described a simple frog method of assay and the present report is concerned with factors that may affect it. The influence of sex, season, repeated injections, starvation and pH have been studied. A comparison has been made of the response of the dog and frog to the same product. Two new preparations of cholecystokinin have been made according to Ivy's S.I. method[6] from dog intestine and compared to the standard previously used. The assay is as follows:

Active dark-colored male frogs weighing 20-40 g are selected, cerebra are crushed, cords pithed and viscera exposed through a paramedian incision. The bloodflow to the gallbladder is observed under a low-power microscope and only those preparations are used that show an active circulation. Injections are made intracardially. The criterion of contraction of the gallbladder is the visual observation of a definite rounding, irregularity or opalescence. When more than one injection is made in the same animal, at least 40 minutes are allowed between injections.

A frog unit is defined as the amount of substance that, when injected intracardially per 30 g frog, brings about contraction of the gallbladder in 50% of 20 or more experiments.

The standard used for comparison is a preparation of S.I. (Ivy) used for experiments previously reported[4] and having a potency of 25 frog units per milligram.

Results and Discussion. Comparison of the gallbladder response of male and female frogs to S.I. injection was made on early summer frogs. Female frogs appear to be slightly less sensitive than males, giving an assay of 20.8 units per mg as compared to 23.7 units per mg for the males.

An analysis of first and subsequent injections in this group of animals shows no appreciable difference in response to the same dosage. Forty minutes or more were allowed between injections in the same animal.

Some seasonal variation in response to S.I. is apparent. Using the same standard preparation, early spring frogs showed an assay potency of 24.4 units per mg; early summer frogs 23.7 units per mg; normal late summer frogs 10.8 units per mg; and fall and winter frogs 21.7 units per mg. Summer frogs that had been starved at room temperature for 3 months presented gallbladders that were markedly distended with thick bile. There were only a few of the gall-

[4] Seager, L. D., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 326.

[6] Greengard, H., and Ivy, A. C., *Am. J. Physiol.*, 1938, **124**, 427.

TABLE I.
Influence of Sex, Season and Starvation on the Assay of S.I.

	% solution	No. of exp.	Contractions	No change
Spring male frogs	.030	40	36	4
	.025	73	55	17
	.020	32	15	17
	.015	29	3	26
Early summer male frogs	.030	20	18	2
	.025	104	72	32
	.020	60	27	33
	.015	32	8	24
Early summer female frogs	.025	98	53	45
	.020	60	26	34
	.015	28	5	23
Starved summer frogs	.025	20	0	20
	.050	24	3	21
	.10	28	4	24
Normal summer frogs	.10	24	15	9
	.05	40	21	19
	.03	24	7	17
Fall and winter frogs	.030	48	38	10
	.025	24	14	10
	.020	28	11	13

bladders of this group of animals that contracted even when large doses of S.I. were used. This finding is in keeping with the observation of McMaster and Elman[5] that with starvation there is a storage and concentration of bile. Failure of these distended bladders to contract is possibly explained by the experiments of Ivy and Doubilet[3] showing that distension of the gallbladder beyond a certain optimal pressure greatly reduced its response to cholecystokinin. Analysis of the above results indicates that with the exception of starved summer frogs and late summer normal frogs, there is no significant statistical seasonal variation.

Two new preparations of S.I. were made from dog duodenum and intestine. S.I.T.$_5$ was prepared as usual by Ivy's method. S.I.T.$_{10}$ was prepared from the filtrate remaining after removal of S.I. from the 5% trichloracetic acid. When the concentration of this acid is brought up to 8-10%, a new precipitate forms which is much finer in texture and settles out more slowly. It was collected on filter paper (Whatman No. 5), washed with absolute aldehyde-free acetone and ether and dried in a desiccator.

Assay of the two preparations showed a potency of 60 units per

[5] McMaster, P. D., and Elman, R., *J. Exp. Med.*, 1926, **44**, 173.

mg for the S.I.T.$_{10}$ and 8.9 units per mg for the S.I.T.$_{5}$. Winter frogs were used.

An attempt was made to rule out histamine-like or choline-like substances as being the active components in these extracts.

0.2 cc of 0.1% atropine sulfate per 30 g frog was injected intracardially in 24 frogs. Subsequent injections of 0.2 cc of 0.1% S.I.T.$_{5}$ gave gallbladder contractions in 23 animals. The dosage of atropine was sufficient to partially or completely block the effect of vagus stimulation on the heart.

That an H-like substance is not an active component of the extracts in these experiments is borne out by the fact that 42 intracardiac injections of histamine of doses varying from 0.1 to 0.4 mg produced no contractions.

Frog saline of varying pH, 5 to 9, produced only one contraction in 82 experiments.

Using the dog method of assay,[1] 5 of 11 dogs showed increased gallbladder pressure on the intravenous injection of 0.2 mg of S.I. per kilo. Weight for weight according to this result, the dog is about 16 times as responsive to S.I. as is the frog. The dog, however, is more expensive as assay material and entails much more labor and time.

Summary. The frog is a satisfactory animal for the assay of gallbladder contracting materials and has the advantage over others of time and expense. Assays on normal summer frogs give lower figures than at other seasons. There is no significant variation in the assays on spring, early summer, male and female frogs, or winter frogs. Starved frogs have gallbladders greatly distended with thick bile. Such distended bladders do not respond well to S.I. The response of the frog's gallbladder to S.I. is not due to the presence of histamine or choline-like substances in the extracts. A modification of Ivy's method for the preparation of S.I. has resulted in the isolation of a more potent preparation and the recovery of much active material lost by the original procedure.