ISOLATION OF SECRETIN AND CHOLECYSTOKININ CONCENTRATES

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ABSTRACT. — Concentrates containing secretin and cholecystokinin have been prepared from a salted acid extract of the upper intestine (A-ppt) of the pig. The latter was extracted with 80% ethanol, the alcohol removed under vacuum and the residue submitted to isoelectric precipitation at pH 5.4 and heating and the activity precipitated from the resulting cooled filtrate (Filt I) by trichloroacetic acid. Filt I was also treated with various adsorbents or chloroform prior to the addition of trichloroacetic acid. Secretin and cholecystokinin in the resulting solids could be further enriched by the addition of amines in aqueous acetone solution. The studies were also extended to other animal species, including those without gall bladder.

Secretin, the hormone stimulating pancreatic secretion and choleresis and cholecystokinin (CCK), the principle eliciting contraction of smooth muscle, notably that of the gall bladder, occur simultaneously in a variety of intestinal extracts. By certain techniques, a preferential enrichment can be afforded. Thus, with the A-precipitate (A-ppt.), a salted acid extract of the upper intestine (Weaver *et al.*, 1926), extraction with media of mounting alcohol concentration progressively decreased the CCK unitage removed (Lueth *et al.*, 1938; Gershbein and Krup, 1952). The reader is referred to several reviews for the earlier literature (Greengard, 1948; Grossman, 1950; Grossman, 1958). According to Ågren (1939), a separation of secretin and CCK was claimed with precipitating agents on the basis of a purported difference in their isoelectric points, being 7.75 and 5.0-5.5 for secretin and CCK, respectively. Gershbein and Krup (1952) showed that the acetone-dried A-ppt (DA) comprises an effective starting material for the isolation of secretin concentrates low in CCK, especially by use of higher concentrations of ethyl alcohol and in conjunction with several other procedures. The hormonal activity of the A-ppt has been precipitated by means of trichloroacetic acid (TCA), alcohol extraction and isoelectric precipitation being employed in the preliminary processing (Greengard and Ivy, 1938). Such products (SI) were further enriched by steps culminating in a "crystalline" secretin picrolonate, the identity of which was later disproved (Greengard *et al.*, 1947). Aside from SI, the occurrence and relative distribution of CCK in the respective fractions derived from this concentrate were not explored by the latter workers.

Fishman (1959) found column chromatography on Dowex 50 and

IRC-50 or paper and starch electrophoresis to be unsuitable in secretin purification starting with a commercial (Lilly) product. Better success was achieved with electrophoresis at pH 7.5, polyvinyl powder serving as the support. By paper electrophoresis, the isoelectric point occurred in the range of 10-11 and a minimal molecular weight of 4400 was deduced on the basis of amino acid analysis. Such determinations have also been carried out by Jorpes and Mutt on their potent secretin products and procedures have been advanced by them for the isolation of the latter as well as pancreozymin-CCK fractions (Jorpes and Mutt, 1955, 1961, 1966; Jorpes *et al.*, 1964; Mutt, 1959).

The present study was undertaken with the view of determining the relative distribution or ratio of secretin to CCK in several concentrates originating from hog A-ppt and to extend such observations to other species. In addition, the purpose was to obtain concentrates containing both secretin and CCK by rather simple techniques toward use in special metabolic and pharmacological studies rather than in the isolation of very active small or simpler molecular types. The assay of secretin and CCK was greatly enhanced by use of the method of isobollic dosages (Gershbein *et al.*, 1949, 1953).

Materials and Methods

The first 3-5 ft of hog small intestine was removed soon after slaughter. The everted strips were freed of any contents and allowed to remain in contact with cold 0.4% hydrochloric acid (200 ml/strip) for 20 min with frequent stirring. They were wrung out by hand and the fluid filtered through cheese cloth and saturated with sodium chloride. Filtration yielded the A-ppt. It was not dried with acetone but used as such. The adsorbents comprised Lloyds' Reagent (Lilly), Permutit (according to Folin), Permutit S, Deacidite 735 and IR-100 (Resinous Products and Chemical Company). The aluminum hydroxide suspension, prepared by the addition of 1% ammonium hydroxide to 1% alum solution according to the Tracy and Welker procedure (1915), yielded 0.75-0.90 g aluminum oxide per 100 g gel on ignition. In general, filtration of concentrates and washing with solvents was achieved by centrifugation. Final drying was carried out over sodium hydroxide pellets under vacuum. The products were stored in the cold.

SI Concentrates

Alcohol extraction of the A-ppt.-The A-ppt was blended in a Waring blendor with 80% ethanol (10 ml/g) and the contents filtered and the residue again extracted with ethanol in the above amount (Greengard and Ivy, 1938). The supernatant fluid was rid of alcohol under reduced pressure at a temperature under 30°C. With the larger batches, the alcoholic suspension was filtered in a Sharples centrifuge and the filtrate freed of alcohol in a glass-lined vacuum still of 100 or 200 l. capacity. The resulting aqueous mixture with a pH generally around 1.5-2.0 was treated with aqueous sodium hydroxide to a pH of 5.5, rapidly heated to boiling with live steam, filtered and the resulting filtrate immediately chilled (Filt. I). The activity was precipitated by the addition of 6N trichloroacetic acid to a final concentration of 5% or after preliminary processing with chloroform or adsorbents. Except for some of the factor studies, the contents were filtered after chilling over a period up to 3 days. The precipitate (SI) was collected by centrifugation, washed with 1.7:1 acetone-ether by volume, 1.7:1 ether-acetone, and finally ether. In several runs, one volume of Filt. I was added to 10-15 volumes acetone and the resulting product after chilling for 24 hr, was filtered by centrifugation and washed with 1:1 ether-acetone portions and finally ether.

Pretreatment with Chloroform.-Filt. I was shaken with 10-20% of its volume of chloroform and the resulting milky suspension broken by a short period of centrifugation; solid occurred at the interface. The top layer was removed and again shaken with chloroform, then

processed with trichloroacetic acid as in the above.

Pretreatment with Adsorbents.—Portions of Filt. I were passed over the respective adsorbent columns and the resulting filtrates were then treated with TCA. Equal volumes of aluminum hydroxide suspension and Filt. I were shaken frequently over a period up to 1 hr before centrifugation; TCA was added to the resulting filtrate. The yellow to amber color of Filt. I was greatly reduced in intensity upon addition of some of the adsorbents, especially the aluminum hydroxide mixture (Al-Susp).

Treatment of SI with Amines and Picric Acid

The procedure of Greengard and Ivy (1938) was employed with modification in the treatment of SI with aniline in aqueous acetone media. The contents were filtered and the aniline precipitate (AP) extracted two more times with the amine mixture. The combined filtrate was freed of aniline and acetone under reduced pressure, several additions of water being required for removal of the free amine. On introduction of the aqueous filtrate into excess acetone, a solid (VII) separated. In a few cases, the aqueous filtrate was filtered after 16 hr of chilling. The aniline precipitate, a source of the hormone pancreozymin, was washed with acetone, 1:1 acetone-ether and then ether. With several SI concentrates, dimethylamine and triethylamine were substituted for aniline. Removal of excesses of these amines was relatively simple because of the lower boiling points. Aqueous solutions of SI on treatment with picric acid in acetone led to the separation of precipitates which could be fractionated by 70% acidified acetone.

Assay Procedures

The method of Gershbein *et al.*, (1949) was employed in the assay of secretin and CCK, 2-3 dogs being used per sample. The pancreatic duct of the pentabarbitalized animal was cannulated and a trocar secured in the dome of the gall bladder after clamping the cystic duct. A sample of hog SI adopted as the standard was arbitrarily assigned a unitage of 1.0 for both secretin and CCK. For secretin, the ratio of submaximal dosages of standard to unknown, each eliciting the same drop rate over a period of 10 min. was equated to the unitage of the test extract. Similarly, the ratio of dosages of either product yielding the same submaximal gall bladder contraction was taken as the CCK unitage of the unknown. For the latter hormone, the *in vitro* assay procedure based on the isolated guinea pig gall bladder was also applied for check purposes (Gershbein *et al.*, 1953); the log dose vs. response curve was widely employed and where the condition of the organ changed, a new curve was derived.

Results

Series A. SI-36.—The processing of 2 kg hog A-ppt by the usual procedure described above yielded 27.73 g of water-soluble powder, SI-36, assaying 4.0 u/mg each of secretin and CCK.

Fractionation with Aniline. — To each of two 500 ml centrifuge bottles containing 4.00 g SI-36 was added with stirring 80 ml water for solution then 320 ml acetone, 0.08 ml concentrated HCl and finally 10 ml freshly distilled aniline. The contents were centrifuged and the supernatant fluid removed. The precipitate was treated with aniline in aqueous acetone two more times and the filtrates combined and concentrated under vacuum; the dried aniline precipitate weighed 3.33 g.

The residual fluid (400 ml) after removal of the free amine was extracted with 280 ml n-butyl alcohol and the emulsion broken by centrifugation at 1500 rpm for 5 min; a negligible amount of interfacial solid resulted. The upper or butyl alcohol layer (BuOH-Ext-I) was set aside and the water layer again extracted with 280 ml of the alcohol to yield the second portion, BuOH-Ext-II. The aqueous solution was rid of alcohol under vacuum and the resulting clear fluid with washings (425 ml) introduced into 5.9 l acetone. A light solid separated after 5 min in

the cold and was filtered two days later; yield of white water-soluble solid, VII-36A: 1400 mg (assay: 12.5 u/mg each of secretin and CCK). BuOH-Ext-I was concentrated under vacuum and the orange-red residue taken up in portions of absolute methyl alcohol totalling 12 ml. On addition of the filtrate to 200 ml anhydrous ether, a solid resulted (BuOH-I-Sol; 188 mg) which assayed for 18 u/mg each of secretin and CCK; the insoluble material (BuOH-I-Insol) weighed 39.4 mg. Under the same conditions, BuOH-Ext II yielded 5.2 mg of essentially inert methyl alcohol-insoluble solid (BuOH-II-Ins.) and 57.6 mg of BuOH-II-Sol (assay: secretin, 11 u/mg.; CCK, $<<11$ u/mg).

Series B. SI Concentrates.—Filt. I in amount of 11.87 l obtained from the processing of 4.0 kg A-ppt. was submitted portionwise to various treatments before the addition of TCA as shown in Table I. An aliquot (3.56 l) was divided among 12 centrifuge bottles and each shaken with 30 ml chloroform. The aqueous layers were separated from the interfacial solid after centrifugation (PR-50; 2.30 g). A second treatment with chloroform produced essentially no solid. The addition of TCA to the filtrate yielded 6.26 g of SI-50.

In the treatment of 400 ml Filt. I with 400 ml Al-Susp, the mixture which was shaken over a period of 1 hr was centrifuged. The clear filtrate (710 ml) on treatment with TCA gave rise to 188 mg of SI-54 (assay for secretin and CCK in u/mg: 9.7 and 9.5, respectively).

Portions of Filt. I in amount of 400 ml were treated batchwise and with stirring over a period of 2 hr with 12 g Lloyds' Reagent, 23 g Zeo-Karb H and 23 g IR-100 and after filtration, TCA was introduced into the respective mixtures and the SI concentrates processed. The yields and assay data are presented in Table 1.

Treatment of SI-47 with Amines. —*Aniline.* Enrichment of hormonal activity of SI-47 was carried out with aniline as per the procedure for Series A except that n-butyl alcohol extraction was deleted. The final filtrate and washings from 2.00 g SI-47 after removal of the excess amine amounted to 72 ml and on introduction into 1.4 l acetone yielded 380 mg VII-46A (assay for secretin and CCK: 9.1 u/mg each).

Triethylamine. Similar to the last experiment, 1.00 g SI-47 was deproteinized in aqueous acetone solution with 5 ml redistilled triethylamine. The final filtrate (36 ml) on treatment with 700 ml acetone gave rise to 249 mg solid (VII-46B; CCK and secretin: 7.6 u/mg).

Dimethylamine. The last run was repeated in all details except that 3.5 ml anhydrous dimethylamine was used in each extraction. The yield of VII-46C was 281 mg and contained either hormonal activity at 7.4 u/mg.

Treatment of SI-50 with Aniline as such and in Conjunction with Al-Susp.-A total of 4.00 SI-50 (secretin: 2.5 u/mg; CCK: 4.0 u/mg) was treated with aniline in aqueous acetone and the final filtrate and washings after concentration under vacuum amounted to 150 ml. Of the latter, 75 ml was introduced into 1.4 l acetone. Yield of VII-50-N: 329 mg. (secretin: 3.0 u/mg; CCK: 11 u/mg). The remaining 75 ml was shaken with Al-Susp (75 ml) over a

period of 35 min and the resulting filtrate concentrated to a volume of 75 ml under vacuum. Treatment with acetone led to the separation of 248 mg of VII-50-CR (assay: secretin, 2.3 u/mg; CCK, 5.9 u/mg).

Series C and D. SA and SI Concentrates.—The use of chloroform and Al-Susp in conjunction with Filt I was further explored in Series C and D (Table 1), employing two additional batches of porcine A-ppt. A second addition of TCA to the filtrate after removal of SI-RIOA precipitated SI-RIOB. The SA products were obtained by adding an aliquot of Filt I to a 10-15 fold excess of acetone and chilling the contents for 24 hr prior to filtration.

The interfacial solid resulting on shaking 100 ml of Filt. I of Series C with 20 ml chloroform and centrifuging amounted to 42.9 mg. The filtrate after removal of SI-R9 when treated with a comparable volume of TCA, yielded 6.9 mg of product (SI-R9B).

SI Concentrates of Series D Employing Combined Chloroform and Al-Susp Treatments.—*Experiments 84T and 85T.* A total of 810 ml Filt. I was shaken with 810 ml Al-Susp over 1 hr after which time the contents were filtered; volume of solution A: 1245 ml. Of the latter, 945 ml was submitted to treatment with TCA and SI-84 removed (208 mg; assay: secretin and CCK, 16.0 u/mg each). The remainder of solution A (300 ml) was shaken with 25 ml chloroform and centrifuged; a minor amount of the interfacial solid was present. The aqueous filtrate on processing yielded 87.0 mg of SI-85 (assay: secretin and CCK: 12.6 u/mg each).

Experiments 86T and 88T. Filtrate I (400 ml) was shaken with 25 ml chloroform and the aqueous layer separated from the interfacial solid (PR-88; 424 mg). Of the filtrate (395 ml: solution B), 95 ml was treated with TCA, yielding 102 mg of SI-88 (assay: secretin, 4.0 u/mg; CCK, 6.8 u/mg). The remaining solution B was shaken with Al-Susp (300 ml) and the final filtrate in amount of 522 gave rise to 30.8 mg SI-90 (assay: secretin, 6.3 u/mg; CCK, 9.6 u/mg).

Further extraction of the A-ppt of Series D.—The A-ppt after the usual two extractions with 80% ethanol was submitted to a third one with 3 l. Processing yielded 5.88 mg SI-77 containing secretin and CCK at 4.0 u/mg each.

Series E. The SA and usual SI concentrates were isolated from Filt. I of yet another collection of porcine A-ppt and the activity compared with the SI products precipitated from the 100 ml aliquots of filtrate passed over columns containing Deacidite 735, Permutit S and Permutit according to Folin. Under these conditions, the latter yielded no precipitate on TCA addition.

Fractionation of SI Concentrates with Picric Acid. Experiment 91P.—SI-36 in amount of 501 mg was dissolved in 20 ml water and treated with a warm mixture of 3.5 g picric acid in 2.5 ml acetone. The resulting precipitate was removed after 16 hr and blended with 20 ml of 70% acidified acetone (composition: 20 ml concentrated HCl, 280 ml water and 700 ml acetone). The filtrate was separated from the solid (P-36-I), introduced into 275 ml acetone and the precipitate washed with acetone un-

til free of picric acid (P-36-S; 286 mg; assay: secretin, 3.3 u/mg and CCK, 0.55 u/mg). The insoluble material, P-36-I, on processing, weighed 123 mg and contained secretin at 1.1 u/mg and CCK at 1.4 u/mg. The overall recoveries of secretin and CCK based on the starting SI were 53 and 16%, respectively.

Experiment 95P.—Similar to the above, SI-47 (499 mg; 1796 u) yielded the soluble product, P-47-S (227 mg; secretin, 2.9 u/mg and CCK, 0.62 u/mg) and the insoluble portion (105 mg; secretin, 1.1 u/mg and CCK, 0.91 u/mg) or a recovery of 774 u (43%) secretin and 237 u (13%) CCK.

Experinment 97P.—The soluble product, P-79-S, arising from SI-79 (386 mg; 2162 u), weighed 215 mg (assay: secretin, 4.0 u/mg; CCK, 0.62 u/mg or recoveries of 40 and 6%, respectively).

Hormonal Concentrates from Other Animal Species. The isolation of SI and allied concentrates from the A-ppt was extended to several other species of either sex. The A-ppt which averaged up to 15 g per upper intestinal strip (3-5 ft lengths) for the hog ranged higher with comparable lengths from cattle and horses. The ratio of secretin to CCK was also close to unity employing the porcine SI standard. From thirteen 15 inch intestinal lengths from rabbits, the yield of SI was 95 mg and assayed 0.57 u/mg each for secretin and CCK. With two collections of beef intestines (22 and 17 strips, respectively), the yields of SI were 413 mg (secretin and CCK: 2.7 u/mg each) and 221 mg (either hormonal activity: 5.3 u/mg), in the order stated. Comparable activities were obtained with canine and ovine SI. Thus, with 30-2 ft intestinal strips from sheep of either sex, the SI concentrate (118 mg; losses) assayed 4.4 u/mg each for secretin and CCK. The yield of SI from 2 macaques (10 inch strips) was 41.0 mg containing each principle at 2.0 u/mg. Data for concentrates obtained from rats, elk and horses, animals without gall bladder, appear in Table 2. All collections were made within 20 min after killing except for a period of 6 hr elapsing between killing of the elk cows in the field and acid extraction of the upper 6 ft of intestine. The everted rat upper intestinal strips were about 2 inches in length. The respective secretin: CCK ratios underwent little change when representative VII-concentrates were prepared.

Discussion

The treatment of the upper intestinal extract, the A-ppt, with two aqueous ethanol portions, removal of solvent under vacuum and isoelectric precipitation at pH 5.5 and steaming of the contents leads to Filt I. In the present study, the latter has been submitted to several fractionation schemes preliminary to precipitation of the activity with TCA to yield the SI products. The crudest concentrates (SA) obtained by addition of Filt I to excess acetone are high in salt and the ratio of secretin to CCK is virtually unity. As the over-all unitage of secretin and CCK in SA is quite close to the values for SI by the conventional method, such solids may be employed in gauging and checking expected activities. Although these concentrates have good

storage qualities, several proved toxic to the assay dog.

In the treatment of Filt. I with TCA to a final concentration of 5%, the SI precipitate forms very rapidly. When the TCA content was increased to 10%, the activities of secretin and CCK did not differ from the conventional SI but a final level of 25% caused destruction of about 30% of either hormonal activity (SI-45; Table 1).

When Filt. I was shaken with chloroform and the suspension broken by centrifugation, removal of the aqueous portion from the interfacial solid and treatment with TCA, yielded SI products in which CCK was definitely higher than the secretin level. The ratios of CCK:secretin amounted to 1.6-2.0. The interfacial solid which displayed an elevation in secretin activity was quite toxic to several of the assay animals. The greatest enrichment of Filt. I was achieved by means of Al-Susp. The resulting fluid after filtration led to SI concentrates with at least three-fold increases in both secretin and CCK activity but with concomitant diminution on a weight or over-all unitage basis. It will also be noted that the 1:1 ratio of CCK:secretin of SI was not altered by the Al-Susp procedure or by deproteinization with aniline, nor on subsequent shaking of the Al-Susp-treated filtrate with chloroform prior to TCA addition. A significant decrease in either hormonal activity was suffered by Filt. I (Series D; Table 1) when the supernatant liquid from the Al-Susp step was submitted to treatment with chloroform or when the order of the two treatments was reversed as summarized in Table 3. In this connection, the addition of Al-Susp to the aqueous residue from SI-50 following removal of aniline, led to a poor yield of concentrate (VII-50-CR) with concomitant diminution in both secretin and CCK as compared to the usual product or VII-50-N; the ratio of CCK to secretin fell from 3.7 to 2.6. These findings lend further support to the contention of the writer that the nature, type and relative distribution of matrix impurities greatly affect the efficacy of various combinations of fractionation procedures. The reader is referred to an earlier article for further illustrations and discussion (Gershbein and Krup, 1952).

The SI-aniline deproteinization procedure of Greengard and Ivy (1938) as modified in this study, gives rise to concentrates (VII) with effective enrichment. They are amenable to clinical application, vasodilatins and pyrogen being essentially absent, at least, at the 'higher' human dosage levels. No pharmacological advantage is afforded by extraction of the aqueous filtrate after aniline distillation with n-butyl alcohol and in fact, such steps contribute toward lower yields. The aniline precipitate by-product contains low levels of secretin and CCK (ratio: about 1) but comprises a good source of pancreozymin, the principle which elicits a pancreatic secretion high in enzyme content. Reactions in several dogs have been encountered in the past on injection of moderate dosages of several of these concentrates. The yields of VII are not unequivocally predictable. The substitution of lower water-soluble amines toward deproteinization of SI has been instituted to circumvent

the rather noxious aniline. Such amines as triethylamine and dimethylamine yield VII-concentrates of somewhat lower but comparable potency and accordingly, show promise in fractionation schemes.

The solid exchange resins and adsorbents used in this study caused little enrichment of hormonal concentrates either by batch or column techniques except for Lloyds' Reagent and Permutit according to Folin. Under the present conditions, Lloyds' Reagent adsorbed virtually all of the activity but this material merits further investigation since the unadsorbed 'inactive' portion constituted about 50% of the conventional SI on a weight basis (Table 1). With Permutit according to Folin, no solid could be recovered after TCA treatment.

Although the recovery of hormonal activity was low, the treatment of the conventional SI in aqueous solution with picric acid and fractionation of the solid with acidified acetone, led to a secretin: CCK ratio which for the portion soluble in this medium was no longer unity, but ranged 4.7-6.6. For the resulting insoluble fraction, the ratio was about 1.

The findings with SI concentrates isolated from various species are of interest. Although the occurrence of CCK has been demonstrated in a number of animals, the relative distribution of the two principles was currently investigated. On the basis of the porcine SI standard, prepared by the usual method and assigned a value of 1.0 u/mg each of secretin and CCK, good correspondence or a similar ratio was deduced with such concentrates from the monkey, dog, sheep, cow and rabbit as was also the case with the products employing Al-Susp and amine pre-treatment. Although the results have been similar with human concentrates, some discrepancies were noted earlier with SI prepared from the intestines of children who succumbed to various diseases, including cystic fibrosis of the pancreas (Gibbs and Gershbein, 1950). In the horse, elk and the rat, species without gall bladder, CCK was unequivocally present and the ratio of secretin to CCK in SI approached unity except for the elk. As both secretin and CCK activities were relatively low in many horse SI concentrates, even in those prepared from intestinal strips removed within 30 min after slaughter, the institution of such procedure as pretreatment with chloroform and Al-Susp, led to enrichment. The diminished levels of CCK often encountered in the horse extracts and the inadequate assay at that time, might have contributed to the observation of Ivy in 1934 that CCK appeared to be absent in this species. As attractive as an hypothesis might have seemed that CCK does not occur in animals without gall bladder, this agent must play a more generalized role in smooth muscle contractility, affecting other organs in addition to the unique action on the circular fibers of the gall bladder (Gershbein *et al.*, 1953; Gershbein and Denton, 1967; Denton and Gershbein, 1967).

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TABLE 1.—Isolation of Salt (SA) and SI Concentrates from Porcine Intestinal Extract, Filt I

Concentrate	Mode of Filt I Treatment[a]	Aliquot, ml	SI			Activity per 1 Filt I u x 10^{-3}	
			Yield, mg	Assay, u/mg			
				Secretin	CCK	Secretin	CCK
Series B (4 kg A-ppt; Filt I: 11.87 l)							
SI-47	Usual procedure[b]	2,600	5,680	3.6	3.6	7.9	7.9
SI-48	Filtration after chilling for 3 hr[c]	400	*A* 870	3.5	3.5	7.6	7.6
			B 162	0.60	0.58	0.24	0.24
SI-49	TCA addition to a level of 25%	400	942	2.5	2.4	5.9	5.7
SI-50	Shaking with chloroform	3,560	6,260	2.6	4.0	4.6	7.0
SI-54	Addition of Al-Susp (400 ml)	400	188	9.7	9.5	4.6	4.5
SI-55	Batch treatment with 12 g Lloyds' Reagent	400	429	0.067	<0.30	.....	.....
SI-56	Batch treatment with Zeo-Karb H (23 g)	400	876	3.3	3.3	7.2	7.2
SI-57	Batch treatment with IR-100 (23 g)	400	954	3.4	3.4	8.1	8.1
Series C (441 g A-ppt; Filt I: 945 ml)							
SA-R6	Salt precipitate with excess acetone	100	5,274	0.052	0.050	2.6	2.6
SI-R8	Usual SI procedure	100	196	2.0	2.0	3.9	3.9
SI-R9	Chloroform	100	156	1.4	2.8	2.2	4.4
SI-R10	Treatment with Al-Susp (200 ml)[c]	200	*A* 65	4.9	5.1	1.6	1.6
			B 45	1.1	2.7	0.25	0.61

TABLE 1.—Isolation of Salt (SA) and SI Concentrates from Porcine Intestinal Extract (Cont'd)

Concentrate	Mode of Filt I Treatment	Aliquot, ml	SI			Activity per 1 Filt I u x 10^{-3}	
			Yield, mg	Assay, u/mg			
				Secretin	CCK	Secretin	CCK
Series D (600 g A-ppt; Filt I: 1515 ml)							
SA-78	Excess acetone	100	3,580	0.35	0.35	1.25	1.25
SI-79	Usual SI procedure	200	478	5.6	5.6	1.34	1.34
SI-84	Al-Susp	615[d]	208	16.0	16.0	0.54	0.54
SI-85	Al-Susp treatment followed by deproteinization with chloroform	195[d]	87	12.6	12.6	0.56	0.56
SI-88	Chloroform	94[d]	102	4.0	6.8	0.43	0.74
SI-90	Deproteinization with chloroform followed by Al-Susp	306[d]	31	6.3	9.6	0.06	0.10
Series E (997 g A-ppt; Filt I: 1620 ml)							
SA-95	Excess acetone	100	7,280	0.25	0.27	18.2	19.7
SI-96	Usual SI procedure	800	3,095	5.8	5.8	22.4	22.4
SI-97	Passage over column of Deacidite 735 (58 g)	100	277	5.8	5.1	16.1	14.1
SI-98	Column treatment with Permutit S (64 g)	100	251	6.0	5.9	15.1	14.8
SI-99	Column treatment with Permutit according to Folin (28 g)	100	—[e]	.....	.....	.....	.....

a. Unless otherwise stated, TCA was added to Filt I as such or after preliminary processing as indicated to a concentration of 5% and the product chilled for 72 hr prior to filtration of the SI concentrate.

b. SI concentrates were similar in yield and hormonal activity where the period of chilling was 48 hr or when TCA was added to a final level of 10%.

c. The filtrate resulting after removal of the *A* concentrate was again treated with the same volume of TCA solution and the *B* product filtered after chilling for 3 days.

d. Yields are calculated on the basis of the volume of the final Al-Susp or chloroform-deproteinized filtrate.

e. No solid resulted even after prolonged chilling.

TABLE 2.—Distribution of Secretin and CCK in SI Concentrates of Animal Species Without Gall Bladder[a]

No. of Intestinal Strips	Filt I Aliquot, ml	SI		
			Assay, u/mg	
		mg	Secretin	CCK
HORSE				
18 (Filt I: 1000 ml)	250; usual SI procedure	186	<0.1	<0.1
	750; Al-Susp	64	0.50	0.50
22	Chloroform treatment	910	1.2	1.8
12 (Filt I: 650 ml)	150; usual procedure	146	0.78	0.78
	500; Al-Susp	179	2.0	2.0
RAT[b]				
24		22	1.5	1.6
82		134	1.9	1.9
21		24	2.0	2.2
24		38	2.0	2.3
30	Chloroform treatment	33	1.3	2.7
ELK				
2		253	<0.1	0.21

a. Unless otherwise stated, the usual procedure as described in the text was employed in the precipitation of SI.
b. The animals of either sex ranged from 150 to 250 g in weight.

TABLE 3.—Summary of Hormonal Activity Distribution and Recovery of Concentrates Treated with Chloroform, Al-Susp and Aniline

Starting Product	Treatment	Assay, u/mg		Ratio: CCK/ Secretin	Unitage Recovered %	
		Secretin	CCK			
SERIES B						
Filt I	Usual SI procedure (SI-47)	3.6	3.6	1.0	.....	.....
SI-47	Conventional aniline treatment (VII-47)	9.1	9.1	1.0	48[b]	48[b]
Filt I	Chloroform (SI-50)	2.5	4.0	1.6	58	90
SI-50	Aniline treatment (VII-50-N)	3.0	11.0	3.7	20[b]	45[b]
SI-50	Aqueous residue after aniline removal treated with Al-Susp (VII-50-CR)	2.3	5.9	2.6	11[b]	18[b]
SERIES D						
Filt I	Usual (SI-79)	5.6	5.6	1.0	.....	.....
Filt I	Al-Susp (SI-84)	16.0	16.0	1.0	41	41
Filt I	Al-Susp followed by chloroform (SI-85)	12.6	12.6	1.0	42	42
Filt I	Chloroform (SI-88)	4.0	6.8	1.7	32	55
Filt I	Chloroform followed by Al-Susp (SI-90)	6.3	9.6	1.5	4	7

a. As compared to that of the respective conventional SI.
b. Unitage recovered based on the starting SI.