に比較して安井，前浜，津呂，中村，河内はいずれも耐性を持ちつつあるといえる．全般的にみて，前浜地域のものについては他地域のものより強い傾向がある．

pyrethroid に対して強い傾向にある地域のものについて新規 pyrethroid の効果を検討し表2の結果を得た．resmethrin, allethrin その他の pyrethroid についても高槻系よりも強い傾向が認められた．しかし，協力剤の混用によって，それぞれ効果はたかまった．したがって，pyrethroid に強い傾向にある点は現状では問題にならないが，今後の課題として検討して置くべき問題である．さらに，調査範囲をひろげるとともに，局地的な検討解析を行う必要がある．なお，継続して調査研究中であるが，北海道と比較して殺虫剤を多量に撒布したと考えられるにもかかわらず，malathion に抵抗性が発達しないことは興味ぶかい点である．

ま と め

高知県下の11地域よりイエバエを採集し，8種類の殺虫剤について感受性の検討を行い，札幌系よりも抵抗性はひくいが，malathion に対して強い抵抗性を持つものがいることが明かになった．また，pyrethroid 系殺虫剤についても稍々強い傾向をもつことが明かになった．

今日まで，高知県下におけるこの種の報告がなかったので現状を報告した．

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Summary

The LD_{50} values of eleven colonies of houseflies collected in Kochi Prefecture are shown in table 1. There was no colony susceptible to γ-BHC, whereas all the colonies tested were susceptible to DDVP, sumithion, and bromophose. Of eleven colonies, three were highly resistant to malathion. Two colonies, whicn were collected in Kiragawa and Murotomisaki, were resistant to malathion, showing 84.826μg and 68.821μg per female as LD_{50} value respectively.

Three colonies, which were collected in Maenohama, Tosashimizu and Yasui, were torelance to allethrin, showing 3.004μg, 1.728μg and 1.937μg per female as LD_{50} value respectively.

Purification and Some Properties of Insect Brain Hormone Extracted from Silkworm Heads

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14. カイコ蛾頭部より抽出された脳ホルモン 宇尾淳子（塩野義研究所） 47. 6. 9 受理

前胸腺刺激ホルモンとしての脳ホルモンの化学的研究については，これまで主としてカイコやサクサン蛹の脳を用いて抽出・精製が行なわれてきたが，生物検定法がそれぞれ非常に異なっているためか，このホルモンの性質さえも判明していない．ここでは，脳ホルモン含量の高いカイコ蛾の，脳を含む頭部全体をホルモン抽出の出発材料とし，信頼度の高い *Samia*-test に重点をおいて，脳ホルモンの精製と検定を行なった．その結果，カイコ蛾1頭分の頭部には，少なくとも50頭のエリサン除脳蛹を成虫化させうるホルモン量が含まれていることが判明した．

種々の溶液による抽出，熱処理，フエノール法，ホルチエ・パーチション法，アクリノールやピクリン酸による沈澱法等，種々の方法でコレステロール，脂質，核酸や或種の大分子物質（糖蛋白，脂質蛋白，ポリサッカロイド等）を除去した後の，主としてペプチッドを含む分画に強い脳ホルモン活性が残った．

この物質の性質について種々の検討を加えた結果，脳ホルモン（活性部分）はこれまでに発表されたような大分子の物質とは考えにくく，分子量5,000以下のペプチッドであると推定される．

Introduction

In 1958 the first active extract from the pupal brain of *Bombyx mori* was prepared by Kobayashi and his collaborators, and later it was crystallized and identified as cholesterol (Kobayashi *et al.*, 1962; Kirimura *et al.*, 1962).

By contrast, Ichikawa and Ishizaki (1961, 1963), who also extracted brain hormone (BH) from *Bombyx* pupal brains found activity in the aqueous

extract; they concluded that the active principle is a protein. Later, they purified their extract by means of heating, ammonium sulfate precipitation, Sephadex gel-filtration and DEAE cellulose chromatography. They concluded that their preparation consisted of heterogeneous polypeptides or proteins with an estimated molecular weight ranging from 9,000 to 31,000 (Ishizaki *et al.*, 1967).

Kobayashi and Yamazaki (1966, 1969) have subsequently confirmed these findings but have not abandoned their steroid theory. They purified BH from *Bombyx* brain by means of chromatography and gel filtration with similar techniques to those of Ishizaki; they concluded that BH may be a glycoprotein containing 15% glucose and having a molecular weight of about 20,000.

At the same time, Williams (1967) confirmed Ichikawa and Ishizaki's results in essence but he hesitated to consider BH to be a protein.

The results of all these extraction efforts agree only on the following points. The active principle is (1) water soluble, (2) heat stable, (3) non-dialyzable, and (4) resistant to some peptidases but not to pronase and Nagase. An important difference in methodology concerns the test species used in the bioassay. Therefore, both *Samia*-(Ishizaki's) and *Bombyx*-tests (Kobayashi's) were routinely employed in the present study. Since the heads of adult *Bombyx* contain a large titer of BH (Ishizaki, 1969) and are easily collected, they were a suitable starting material, although they contain more unnecessary components than isolated brains do.

The present paper deals with the results of the extraction and purification of BH from the insect head. After removal of nucleic acid, lipid, lipoprotein, glycoprotein, and cholesterol, a fraction containing mainly peptides had a strong brain hormone activity. Contrary to the findings of former investigators, the outer solution as well as the inner solution after cellulose-tubing dialysis showed substantial activity in the bioassay.

Materials and Methods

Source of BH extract

Since brains of *Bombyx* larvae, pupae, and adults are all hormonally active (Ishizaki and Ichikawa, 1967), and BH titers in the heads of newly emerged adults are rather high (Ishizaki, 1969), *Bombyx* heads collected from moths one day before to one day after emergence served as starting material. The 255,000 insects used were randomly selected and not of a specific race. The heads were kept frozen until extracted.

Bioassay of BH

Bioassay of BH was performed on brainless pupae of Eri silkworms, *Samia cynthia ricini* (Ishizaki and Ichikawa, 1967) at any time between 2 and 8 months after removal of the brain, and on the debrained "dauer pupae" of a special strain (J122×C115) of silkworm, *Bombyx mori* (Kobayashi, 1957; Kobayashi and Yamazaki, 1966). In the latter case, test animals were limited to males, and the injection time was strictly limited to from 4 to 5 weeks after brain removal (Nishiitsutsuji-Uwo, 1971). For the assay in *Samia* 19,000 insects were used, and for the *Bombyx* assay 15,700 insects.

The test solutions were injected into assay pupae at a dorso-lateral site of the 4th abdominal segment. Each pupa routinely received 0.04m*l* (occasionally 0.02-0.08m*l*) of the test solutions. Animals were kept at 25°C throughout the experiment.

In the present study, 5 test-pupae of *Samia* and 10-12 tset-pupae of *Bombyx* were routinely used for the assay of each experimental lot. BH activity was measured by assaying a series of 3-4 fold diluted solutions of the preparation. Bioassays were usually made by double tests with *Samia* and *Bombyx* or repeated twice with different *Samia* pupae reared at different times. Observations were made every other day for 4 weeks after the injection. Specimens died or emerged within 10-12 days were excluded from the available numbers.

Penicillin and phenylthiourea were occasionally added to the injection following the method of Ishizaki and Ichikawa (1967) when preparations for injection were very crude. *Samia*-unit of BH was determined following the same authors.

Chemical procedure

The chemical procedures were carried out below 4°C, and preparations were keept frozen. Protein concentrations were determined by Lowry's

method (Lowry *et al.*, 1951), DNA by Burton's method (1956), RNA by the orcinol-HCl method (Mejbaum, 1939), and sugar by the anthrone method (Scott *et al.*, 1953).

Results

Acetone powder (*AP*)

Adult heads (fresh weight; 9 g per 1,000 insect heads) just before to just after emergence were homogenized in over 20 times their volume (v/w) of cold acetone (-10°C) and the inactive supernatant was discarded. This procedure was repeated until the supernatant was colorless. The pellet was dried under reduced pressure and stored frozen. In abbreviated form this homogenase is called *AP*. 2.2g of acetone powder is obtained from 1,000 heads.

Crude extract (*CE*)

Active material was readily extracted either from the fresh head or from the acetone powder with aqueous solutions such as 2% NaCl, 0.3M NH_4Cl, 0.3M $HCOONH_4$, 0.05-2M acetic acid, 0.85% NaCl in 5mM phosphate buffer, 0.2M phosphate buffer, 0.05-0.2M Tris-HCl, 0.05-0.2M pyridine-acetate etc. whose pH, except that of acetic acid, were adjusted to 5.0-7.6. Extracts prepared in 2% NaCl or 0.2M pyridine-acetate, pH 6.0, showed the best results both in *Samia* and *Bombyx* tests. The procedure of freezing and thawing the AP with extraction solution was useful to obtain better BH titers. These extracts are designated as *Crude Extract* (*CE*).

Heat treatment (*HF*)

A small amount of crude extract was placed in a large flask and shaken at 90°C for 3 minutes. After rapid cooling in ice cold water, all activities remainad in the saline supernatant, thereby confirming the heat stability reported by former investigators. Since many inactive components were removed in this step, heat treatment was invariably done.

Folch partition (*Ch. Me upper*)

In order to remove lipophilic fractions, Folch partition (1957) was applied to the preparation: four volumes of a chloroform: methanol (2:1) mixture was added to one volume of the preparation. After vigorous agitation, the lower (chloroform) layer was back-washed once and discarded. The upper layer was washed once with chloroform, then combined with the washings of the lower layer and lyophilized. This fraction is abbreviated *Ch. Me upper*.

Phenol treatment (*phenol lower*)

Crude extract from acetone powder contains little DNA but a considerable amount of RNA and sugar. The principle of Dixon and Stack-Dunne (1955) was adopted as follows: preparations were re-extracted once with the same volume of phenol saturated with water and once with half the volume of the same solution. The phenol extracts were washed several times with water. The aqueous phase contained macro-molecular components such as nucleic acid, lipopolysaccharide and macroglycoprotein etc. This phase was repeatedly washed with ether to remove phenol and called *phenol upper* although this phase had no BH activity. To the phenol phase including the intermediate phase (both phases had BH activity and it was difficult to divide them) water and diethyl ether were added. After equilibration the aqueous phase was removed and the ether-phenol phase was back-washed with water. The principal aqueous phase and the washings were combined and repeatedly washed with ether in order to remove all traces of phenol. This fraction is designated as *phenol lower*, and contains a high titer of BH.

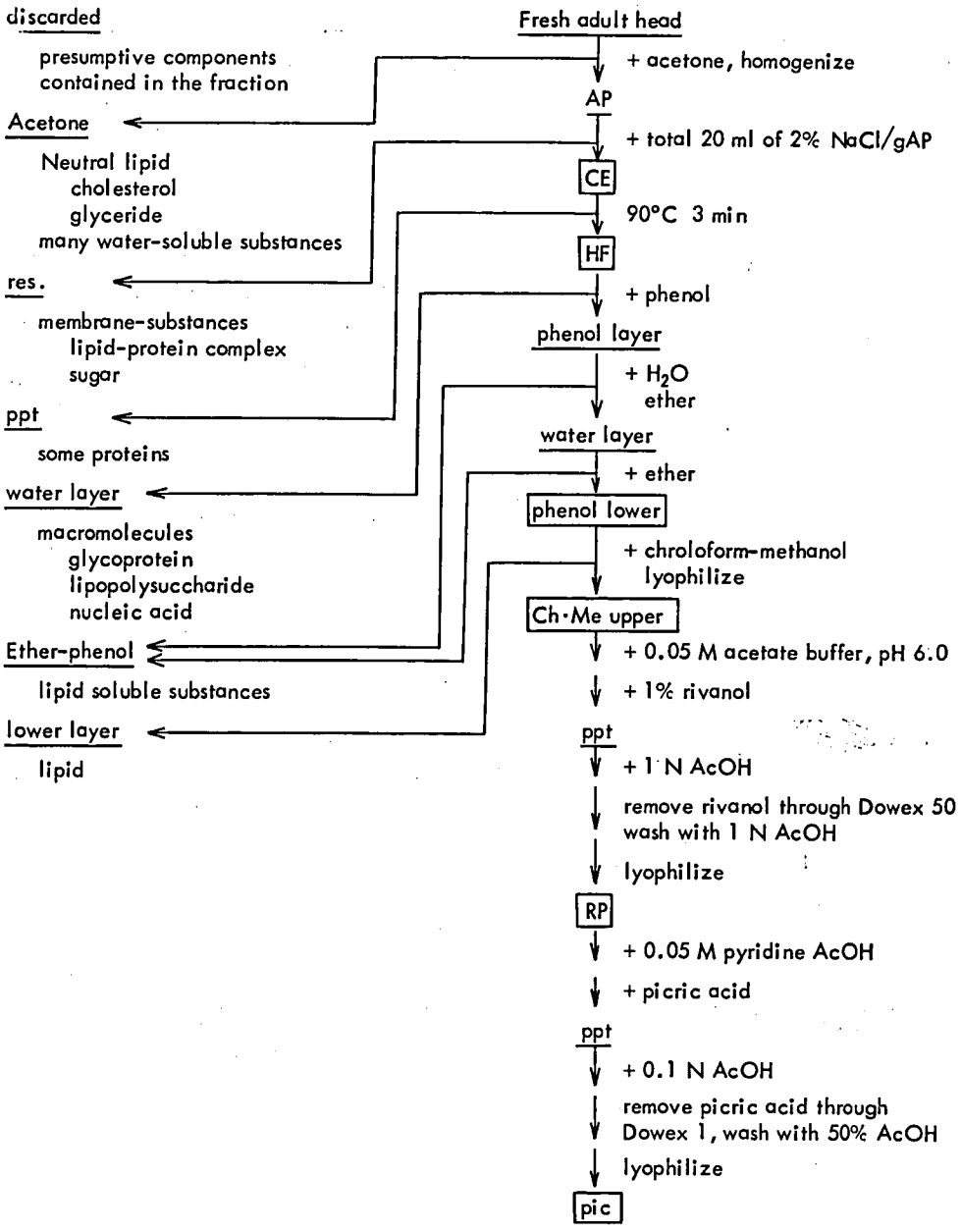
Rivanol precipitation (*RP*)

The brain hormone can be precipitated by acrynol (Rivanol ®, 6, 9-diamino-2 ethoxyacridine; Hoei-Yakuko & Co., Ltd., Japan). When 0.1-1% acrynol aqueous solution (final concentration 0.07-0.3%) was added to lyophilized *phenol lower* dissolved in a 0.05M acetate buffer pH 6.0, the precipitate retained all of the BH activity, the supernatant having none. When this lyophilized *phenol lower* was dissolved in 2.5M acetate buffer solution, the precipitate contained no BH activity. Removal of acrynol was done either with CM cellulose or with Dowex 50×2. This precipitate fraction from 0.05M acetate buffer pH 6.0 is called *Rivanol Precipitate*, or *RP* in abbreviated form.

Precipitation with picric acid (*Pic*)

Active extract is also precipitated by picric acid. Saturated aqueous solution of picric acid

Fig. 1. Schematic diagram showing an example of procedure for extraction and purification of the brain hormone extracted from silkworm heads.



was added to a solution of the active fraction. Excess picric acid was removed by adding Dowex 1-×2 to a solution of the precipitate in 1 N acetic acid. The filtrate and washings with 50% acetic acid contained a good BH activity. This fraction is called *Pic*.

Six steps of extraction and purification procedures

Based on the results mentioned above, the BH extracted from the heads of adult silkworms was partially purified. Extraction and purification were carried out by successively removing unnecessary components. A summarized example of these procedures is shown in Fig. 1.

The BH-titer, protein-(or peptide), RNA-,

and sugar-contents of the 6 preparations, converted into values for one adult-head, are given in Table 1. An adult head contains at least 50 *Samia* units. Although yields of protein, RNA and sugar decreased by 96%, 99.6% and 99% respectively with the progress of the purification, BH activity (*Samia* unit) had a good yield of 65%. As the purpose of these methods was not to separate the peptides but principally to remove unnecessary components, the final preparation, *Pic*, still contained considerable amounts of peptides or proteins other than BH, evidenced by the appearance of many bands stained by Amidoschwarz 10B when disc electrophoresis on 15% acrylamide gel (pH 8.3) was applied. Nucleic acids and sugars, however, were markedly reduced.

Characteristics of BH

The nature of each preparation with BH activity was checked by the following experiments.

A) Effect of dialysis.

Phenol lower was used as a test sample. A half millilitre of extract, diluted to 1 m*l* with water, served as a positive control (A). Next, 0.5m*l* samples were diluted to 1m*l* with either 0.05M tris-HCl, pH 7.6 (B), or 0.05M acetate buffer containing 0.1M NaCl. pH 6.0 (C) and dialyzed against 10m*l* of the respective buffers, first at 20°C for 3 days and then at 4°C for 1 day. After dialysis, the volume of the bag-solution was adjusted to 2m*l* (B1 and C1) and the outer solution was concentrated to 1m*l* (B2, C2). In addition 10m*l* of each buffer was also concentrated to 1m*l* and served as a negative control (B3, C3). The results are seen in Table 2. The *Samia*-test showed that the inner solutions, B1 and C1 retained a level of BH activity similar to that of the control A. The outer solutions, however, had good BH activity, in contradiction to the findings of the other 3 groups of investigators who agreed that BH is a non-dialyzable material. One third of the BH activity seemed to pass through the cellulose tubing. The ten-times condensed buffers had no effect on the brainless *Samia* pupae.

B) Effects of ions.

To each of 0.5m*l* of 0.1M solutions of sodium acetate, potassium acetate, pyridine acetate, tris-acetate, calcium acetate, magnesium acetate, and cupric acetate (whose pHs were adjusted to around 6) was added 0.5m*l* of the *phenol lower* preparation. Each solution was then dialyzed against 15m*l* of its corresponding 0.05M solution for 3 days at 20°C. Thereafter, each solution was dialyzed against 150m*l* of 0.05M tris-HCl pH 7.6 for 1 day at 4°C.

Table 1. Purification of BH extracted from 1 head of adult silkworm.
—data calculated on a "per head" basis—

Name of preparation	CE	HF	Phenol lower	Ch. Me upper	RP	Pic
*BH (Samia unit)	54	44	36	40	46	35
Yield of BH (%)	100	81	67	74	85	65
Protein (μg)	912	438	196	203	56	38
Yield of Prot. (%)	100	48	21	22	6	4
Effective doses (μg)	17	10	5.4	5.3	1.2	1.1
**Specific activity	59	100	184	197	821	921
Purification fold	1.0	1.7	3.1	3.3	13.9	15.6
RNA (μg)	107	96	—	—	0.8	0.4
Yield of RNA (%)	100	90	—	—	0.7	0.4
RNA/prot.	0.12	0.22	—	—	0.014	0.011
Sugar (μg)	38	37	—	1.8	0.7	0.4
Yield of Sugar (%)	100	97	—	5	2	1
Sugar/prot.	0.042	0.084	—	0.009	0.013	0.011

* One *Samia* unit of BH was defined as the minimum amoumt necessary to cause adult development in one assay pupa.

** Specific activity: BH units/mg protein (Lowry positive material).

Table 2. Effect of dialysis.
BH sample, *phenol lower*, was dialyzed against a buffer for a total of 4 days. After dialysis, solutions inside and outside of the dialysis tube were bioassayed.

	Sample		*Samia*-test	Total prot. mg	Recovery of prot.	
A	BH extract	no dialysis	++	12.7	100	
B_1	0.05 M Tris-HCl	inner sol.	++	6.4	50.3	103%
B_2	〃	outer sol.	+	6.7	52.4	
B_3	buffer only		−	0		
C_1	0.15 M Na-Acetate	inner sol.	++	7.2	56.6	92%
C_2	〃	outer sol.	+	4.5	35.6	
C_3	buffer only		−	0		

A: Extract without dialysis.
B_1 and C_1: inner solution after 4 day-dialysis.
B_2 and C_2: outer solution after 4 day-dialysis, concentrated 10 times with evaporator.
B_3 and C_3: buffer employed for dialysis, concentrated 10 times.

Table 3. Effect of ions on BH activity.
0.5m*l* samples of *phenol lower* were added to solutions of the ionic compounds (0.5m*l*; final conc. 0.05M or 5M) then dialyzed; first against the same solutions for 3 days at 20°C, then against 0.05M tris-HCl pH 7.6 for 1 day at 4°C.

Procedures	Protein total mg	Recovery of prot.	*Samia*-test
0.05M Tris-HCl (control)	0	0	−
phenol lower 2 (original)	6.5	100%	++
dialysis against H_2O	2.7	42	−
〃 0.05M CH_2COONa	2.2	34	+
〃 0.05M CH_3COOK	2.2	33	++
〃 0.05M Pyr-AcOH	2.8	43	++
〃 0.05M Tris-AcOH	2.4	38	+
〃 0.05M $Ca(CH_3COO)_2$	2.4	38	±
〃 0.05M $Mg(CH_3COO)_2$	2.1	32	−
〃 0.05M $Cu(CH_3COO)_2$	2.9	44	+
〃 5M LiCl	4.6	71	++
〃 5M NaCl	7.0	108	+
〃 5M CsCl	4.8	74	+
〃 6M Urea	4.8	74	+

As seen in Table 3, BH activity of the inner solution of the bag was retained following dialysis against the solutions containing potassium and pyridine ions; less activity was seen with sodium, tris, and copper ions.

Dialysis against deionized water only, or against solutions containing calcium and magnesium ions had an adverse effect upon the BH activity.

C) Effects of high concentration of the salts.

The same procedure followed in B above was done using 5M-LiCl, -NaCl, -CsCl, and 6M urea instead of 0.05M solutions. Little or no effects on BH activity appeared under such high salt concentrations, and the recovery of protein was higher than in the B series (Table 3).

D) Enzymatic digestion.

Enzymatic digestion tests were often carried out on the preparations from the different steps of the purification. Enzymes, at concentrations of 1/50 of the concentrations of protein in preparations, were added to the samples, which were then kept at 37°C for 5-6 hrs. The hydrogen exponent of each sample solution was adjusted to the optimum for its enzymatic reaction. Blank tests were done by adding the same volume of solution without enzyme to the preparation:

Table 4. Digestion of BH preparations by proteolytic enzymes.

*Procedure		*Samia*-test	*Bombyx*-test
Preparation 1.	no enzyme	++	+
	+ trypsin	+	+
	+ thermolysis	+	−
Preparation 2.	no enzyme	+	++
	+ trypsin	±	−
	+ thermolysin	±	±
Preparation 3.	no enzyme	++	
	+ Ribonuclease	++	
	+ thermolysin	++	
	+ Nagase	−	
Control	2% NaCl	−	−
	0.05M pyridine-AcOH	−	

* Preparation 1: AP → CE by 2% NaCl → HF
Preparation 2: AP → CE by 0.05M pyridine-AcOH → HF
Preparation 3: *Ch. Me upper*
'No enzyme' means incubation at 37°C for 5-6 hrs without enzyme.

Some examples of the results are illustrated in Table 4.

There was a complete loss of BH activity after incubation with Nagase (commercial name of crystalline subtilisin, supplied by the Nagase Co., Ltd.) or pronase (the proteinase purified from *Streptomyces griseus*, supplied by the Kaken Co., Ltd.), but no effect was seen with ribonuclease. The BH preparations were generally resistant to peptidases such as trypsin, chymotrypsn, and thermolysin, though there were some exceptions as seen in preparations 1 and 2 of Table 4.

E) Gel filtration on Sephadex G 25.

Although pioneer workes confirmed that BH was completely excluded from the particles of Sephadex G 25 (Ishizaki *et al.*, 1967) and G 50 (Kobayashi *et al.*, 1966), in the present work BH was found to be not completely excluded from even sephadex G 25 (see Table 5 Example 1). This result, as well as the dialysis results (Table 2), suggests that an active core of BH, or a component of BH contained in a heterogeneous substance, might be a small molecule whose molecular weight would be less than 5,000.

F) Ion exchange chromatography.

Many attempts were made with various kinds of ion-exchange celluloses for purification of BH. Unfortunately, the assay results were not satisfactory although various conditions of pH and molarity of elute-solutions and starting samples were employed. Table 5 shows some examples of the results of chromatographies on DEAE-and CM-celluloses: Different results of bioassay with *Bombyx*-test and *Samia*-test were often seen (Table 6). If one considers the results of the *Samia*-assay only, BH activity appears both as a bounded and a non-bounded fraction on CM cellulose, and as a bounded fraction only on DEAE cellulose. Similar results were obtained with Dowex 50−×2 and Dowex 1−×2. If BH is regarded as a heterogeneous substance, then the present results, though contrary to those of Kobayashi *et al.* (1966), may not be contradictory to those of Ishizaki *et al.* (1967). Details of ion exchange chromatography of BH will appear in following papers.

Discussion

Attempts to extract BH from whole heads of the silkworm instead from their brains only, and to remove many unnecessary components have been made successfully. Heads of newly emerged adults contain a high titer of BH. However, contamination with pigment often interferes with separation of the components. The present work mainly deals with removal of unnecessary components.

The head of an adult *Bombyx* contains at least

Table 5. Some examples of chromatography under various conditions on G 25 and ion-exchange celluloses.

Two samples, either *RP* or *Pic*, were employed for the chromatographies. Extraction steps were: AP → CE → HF → phenol lower → Ch. Me upper → RP → Pic

Example No.			
1	Sephadex G 25 (1.5×50cm column, elution by 0.2 N AcOH, sample, 2m*l* of *Pic*)		
	Fractions	Yield of prot.	*Samia* test	Effective doses
	fraction 1 excluded	51%	++	0.8μg
	fraction 2 between fractions 1 & 3	9%	++	1.0
	fraction 3 same fraction Nos. of NaCl eluted	3%	+	4.0
2	CM cellulose (1.4×12 cm column, stepwise, sample *Pic*)			
	1. 0.05M pyridine-AcOH pH 4.5	51%	++	1.3
	2. 0.2M 〃 〃	33%	++	0.9
	3. 0.5M 〃 〃	3%	±	
3	CM cellulose (1.4×5cm column, two steps, sample *RP*)			
	1. 0.05M Acetate buffer pH 6.0	26%	++	1.0
	2. 1 N NaCl in buffer	33%	++	>0.9
4	DEAE cellulose (1.4×12cm column, stepwise, sample *Pic*)			
	1. 2M pyridine pH 8.8	18%	±	
	2. 〃 pH 7.0	3%	−	
	3. 〃 pH 5.2	31%	+	2.0
5	DEAE cellulose (1.4×5cm column, two steps, sample *RP*)			
	1. 0.05M Tris-HCl pH 7.6	10%	−	
	2. 1 N NaCl in buffer	42%	++	>1.2

Table 6. Details of bioassay of examples 3 and 5 in Table 5.

*×1 .04	×4 .04	×16 .04	×64 .04 m*l*	Judgement	Name of preparations	Prot. mg/m*l*	×1 .04	×4 .04	×16 .04	×64 .04 m*l*	Judgement	Effective doses/ Samia
	Bombyx-test						*Samia*-test					
**1.8	1.7	2.4	0.7	−	2% NaCl (control)	0	0.5	0.5	0.5	0.5	−	
4.3	3.6	0.6	1.8	+	RP original	0.9	5.0	5.0	5.0	1.4	++	1.2μg
3.1	1.5	0.6	3.4	+	CM-1 not bounded	0.8	4.1	4.1	3.2	1.4	++	1.0μg
2.5	1.6	0.6	0.9	−	-2 bounded	1.5	4.1	5.0	5.0	4.1	++	>0.9μg
1.7	0.3	2.7	2.7	−	DEAE-1 not bounded	0.3	1.4	2.3	1.4	0.5	−	
2.4	3.7	2.5	0.8	−	-2 bounded	1.9	5.0	5.0	5.0	4.1	++	>1.2μg

* × means dilution-fold, in this case, no more dilutions of 64-fold was performed.

** large figures at left: number of specimens developed into adult.
small figures at right: number of specimens unchanged.

50 *Samia* units of BH. Fractions containing mainly peptides retain a high titer of BH activity after the removal of lipid, cholesterol, main structural protein, macromolecules including glycoprotein, lipopolysuccharide, and nucleic acid.

The most interesting finding is that BH activity appeared even in the solution outside of cellulose tubing after dialysis. The result of gel-filtration on Sephadex G 25 also suggests that some component(s) of a heterogeneous BH, or alternatively an active core of BH seems to be a peptide (s) of 5,000 daltons or less.

Ishizaki and Ichikawa (1967) reported that BH consists of small proteins (or peptides) and manifests highly heterogeneous molecular forms whose estimated molecular weights range from

9,000 to 31,000. Since they usually dialyzed the preparations, and performed further purification with the inner solution only, small molecular substance (s) having BH activity would have escaped. Yamazaki and Kobayashi (1969) estimated the molecular weight of the brain hormone extracted from the same *Bombyx* pupal brain as used by Ishizaki to be about 20,000, and they thought it to be a glycoprotein containing 15% glycogen. The present material after treatment with phenol, cannot contain glycoproteins having a large molecular weight such as 20,000. Gersch and Stürzebecher (1968) also reported that besides Neurohormone D, the two active factors of BH were extracted from *Periplaneta americana* which seemed to be proteinic with molecular weights of 20,000 to 40,000.

On the contrary, Williams (1967) hesitated to conclude that the BH is a protein because of (1) its heat-stability, (2) its lack of specific absorption at 280 mμ, (3) its insensitivity to pepsin, trypsin, and chymotrypsin, and (4) its insensitivity to classical methods used to remove the last traces of proteins. According to Williams, there is at least a possibility that the BH of insects might turn out to be a mucopolysaccharide instead of a protein or a protein-containing complex. It would be difficult to assume that BH is a mucopolysaccharide, because, (1) the present extract after removal of polysaccharides has a strong BH activity; moreover, (2) the histochemical as well as chemical approach has yielded at least strong circumstantial evidence in support of the concept that the majority of active neurosecretory mediators in invertebrates, as well as in vertebrates, are peptides (Scharrer *et al.*, 1970).

Kobayashi and his colleages (1966) conclude that the brain hormone consists of 'steroid and proteinic substances'. Cholesterol is known to be ubiquitous in the tissues of diapausing pupae (Schneiderman and Gilbert, 1964) and its seemed incredible that a few additional molecules of this same substance could function as the brain hormone (Scharrer *et al.*, 1970).

Papers dealing with the test animals for the biological assay and with further purification of the brain hormone will appear in the near future.

Summary

Attempts to extract the prothoracotropic hormone from the head of *Bombyx* involved the removal of unnecessary components such as cholesterol, lipid, nucleic acid, lipophilic substance and certain macromolecules including glycoprotein, lipoprotein, and polysaccharide. The resulting partially purified brain hormone was shown to be highly active. The head of an adult silkworm contains at least 50 *Samia* units of brain hormone. This hormone seems to be a peptide-like substance. It is water soluble, heat stable and resistant to certain peptidases, but its active core appears to be dialyzable.

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Studies on Pyrethroidal Compounds* Part III Photostability of Pyrethroidal Compounds Yasuo Abe, Kosuke Tsuda and Yoshio Fujita (Research Department, Pesticides Devision, Sumitomo Chemical Co., Ltd., Takatsukasa 4-2-1, Takarazuka City, Hyogo, Japan.) Received June 21, 1972. *Botyu-Kagaku*, **37**, 102, (1972).

15. ピレスロイド系化合物の研究 第3報 ピレスロイドの光安定性

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合成ピレスロイド4種および天然ピレスリン類6種の光安定性について調査し，次のことを明らかにした．

1. 合成ピレスロイドは天然ピレスリン類に比べて一般に安定である．2. Resmethrin が最も安定であり，Tetramethrin と Furamethrin がそれに次ぐ．Allethrin はやや不安定である．3. 天然ピレスリン類6成分の中では Pyrethrin Ⅰと Ⅱが極端に不安定である．4. Cinerin Ⅰと Ⅱは Pyrethrin Ⅰと Ⅱより安定であり，Jasmolin Ⅰと Ⅱは，それぞれ Cinerin Ⅰと Ⅱ及び Pyrethrin Ⅰと Ⅱの中間くらいの安定性である．5. 第一菊酸エステルは第二菊酸エステルより光に対して不安定である．更にピレスロイドの紫外吸収極大波長と安定性との関係について述べた．

B.H.T. 及び種々の芳香族アミンのアレスリンに対する光安定化効果を調査し，N,N'-Diphenyl-*p*-phenylenediamine が最も効果が強いことを明らかにした．蚊取線香中ではアレスリンは，光の影響は小さいことがわかった．

更に天然ピレスリン類の中で Pyrethrin Ⅰ及び Ⅱのみが特異的に不安定な原因について考察した．

Introduction

In spite of their relatively high cost as compared with other insecticides, the current increase in demand for insecticides which do not leave toxic residue in plants or animal foodstuffs for human consumption has resulted in renewed attention to pyrethroidal insecticides. Pyrethroids

* The previous paper, *Botyu-Kagaku*, **37**, 48 (1972).