The Cardio-tonic and Diuretic Action of *Kobu* (*Laminaria Japonica*, Aresch).

By

MASAO WATANABE.

(渡邊正雄)

(From the Pharmacological Laboratory [Prof. S. Yagi],
Tohoku Imperial University, Sendai.)

Laminaria japonica Aresch, the Japanese classical appellation *hirome* or *ebisune*, popularly called *kobu* is the product of the adjacent seas of Hokkaido or Ou provinces. This plant grows on the rock or the sand at the bottom of the sea. Its stalk is from one to four inches long; its upper part becomes flat and passes to the blade. The blade is long belt-shaped, with round base, and gradually narrows toward the apex; seven or eight feet in length, from seven inches to one foot in breadth. This plant contains iodide, mannit, protein and fibrous matter; of the other components little is known.

The blade of this plant has been widely used for food in this country and in China and is sometimes used as a cardio-tonic among the people. On the pages concerning *kobu* of "Honzokomoku" (a Chinese classical pharmacopoeia), we read that this plant has some curative effect upon oedema of various origins. If that is the case, it might be that this plant dissipates oedema indirectly by its cardio-tonic action as popularly believed, or that it has a special diuretic action, or both of those actions. It seems rather strange to think that this substance which is used as a daily food stuff should contain the components which have a cardio-tonic and diuretic action, a thing which needs thorough investigation.

For instance, when a decoction prepared from the dried *kobu* sold in the market was given to a frog, it was found that its heart beat became stronger, and when given to a rabbit, the secretion of urine increased, though these actions were very slight. But can we not expect

a more remarkable effect, if its efficient components were isolated and given to animals? It has now been attempted to isolate such an effective component by simple chemical manipulation and to investigate its cardio-tonic and diuretic action. In the present paper the result of this investigation is briefly described.

ACTION ON COLD-BLOODED ANIMALS.

(1) The action of *kobu* decoction.

(1) *General status.* 10 % decoction of powdered *kobu*, neutralized with sodium carbonate, was introduced in the abdominal lymph sac of *rana temporaria* and the effect upon the general status was observed.

When the decoction was given to the frog in a dose of 0.7 c.c. or more per 10 grms. of the body weight a remarkable change occurred. The spontaneous movements and respiration became gradually infrequent and slower, until they finally disappeared. The animal jumped on a mechanical irritation awkwardly; when it was turned on its back, it tried to regain the former position. But such reaction soon disappeared. According to the dose administered, it gradually recovered or died. The contraction of gastrocnemius muscle resulting from the electric stimulation of an exposed sciatic nerve remained normal even after all voluntary and reflex movement had disappeared.

(2) *Heart.* A *rana temporaria* was laid on its back and its heart exposed in the usual manner, and into the lymph sac of the thigh 10 % *kobu* decoction was injected in a dose of 0.7 c.c. per 10 grms. of the body weight. The systole and diastole of the heart became gradually stronger, now and then peristaltic movements arose, and frequently after a few hours systolic or half systolic standstill followed. Even if we increased the amount administered there was not much difference of time needed for bringing about the standstill. But when a small quantity of this decoction, for instance 0.1 c.c., was intravenously introduced, the above described symptoms immediately appeared, an evidence that the decoction passes very slowly from the lymph sac to the blood.

The heart of the frog was excised and perfused according to the Straub's method. Addition of a drop of this decoction into the perfusion fluid gave rise to a remarkable increase of contraction and dilation, but further drops brought on diastolic standstill after preliminary increase of amplitude (Figs. 1 and 2).

Fig. 1. Excised heart of *temporaria*. One drop of 10 % *kobu* decoction. Time marks in 5 seconds.

Fig. 2. Excised heart of *temporaria*. Five drops of 10 % *kobu* decoction. Time marks in 5 seconds.

(3) *Blood vessels*. When 0.25 c.c. of the decoction of a concentration stronger than 0.5 % was injected into the inflow cannula of frog's thigh perfused according to the method of Trendelenburg, a temporary but distinct decrease of the outflow was observed, the rate of the decrease being proportional to the concentration of the decoction.

From the above described experiments, the *kobu* decoction seems to act on the frog in the following ways :

1. It abolishes the voluntary and reflex movements of the animal paralysing the central nervous system.
2. It acts on the heart, augmenting the contraction and dilatation and finally bringing it to a nearly diastolic standstill.
3. It acts on the wall of the blood vessel constrictively.

It is not clear whether or not all these actions are due to the same substance present in the *kobu* decoction. Its action on the heart greatly resembles the action of digitalis, but in the last period of the poisoning, unlike the action of digitalis, it brings a standstill in diastole. Is this not because the *kobu* decoction contains in addition to the digitalis body, a substance belonging to one which would paralyse the motor apparatus of the heart? In order to answer this question, attempt was made to isolate the active principles of *kobu* by using some solvents, and to investigate their action separately.

(II) The action of the components of *kobu*.

By extracting crushed *kobu* with 95 % alcohol under heart, a dark greenish acid fluid was obtained. The alcoholic extract was evaporated

on a water bath, water added, stirred and neutralized with sodium carbonate. The extract thus treated had the same action as the decoction already described, while the decoction obtained by adding water to the residuum of the extract had no action on the heart, though it paralysed the central nervous system. It follows then that the cardio-tonic substance had all passed into the heated 95 % alcohol. When the alcoholic extract was filtered while it was still warm and then cooled, large radial crystals were precipitated. Afterwards the crystals were filtered, the filtrate evaporated by heating on the water bath, some absolute alcohol added to the residuum, stirred, filtered and evaporated again. By repeating such manipulations several times a dark greenish concentrated extract was obtained. This extract having been mixed with ether, stirred and filtered, washed several times with water and evaporated, there remained a clear greenish colored dense fluid, which was slightly fragrant but had no taste. It was almost insoluble in water, but dissolved very easily in alcohol, ether, petroleum ether, chloroform and benzol. For convenience, we call this "‘A’ substance." When the above mentioned part which is insoluble in ether was washed again with ether and dried, we got a brownish colored lump, which had a resinous smell and taste and was soluble in water and alcohol giving an acid reaction. In order to distinguish this substance from the former, it is called "‘B’ substance." The crystals filtered from the alcohol extract were dissolved in heated 95 % alcohol and recrystallized. After repeating this manipulation, we obtained large radial crystals which had a pure white silk-like lustre, a sweet taste, were odourless and soluble in water. These and other characteristics suggest that this substance is nothing other than mannit. The experimental study of these three components yielded the following results.

(1) *The action of "A" substance.* Since this substance was nearly insoluble in water, I prepared 10 % alcoholic solution, and in using it I emulsified the solution with water or Ringer's solution.

(i) When 1 c.c. 1 % emulsion was injected into the abdominal lymph sac of rana temporaria of middle size no recognizable symptom appeared. After a few hours the breast was opened and the heart examined. Generally the heart beats were slow, the contraction of the ventricle was strong while its dilatation was imperfect.

(ii) A rana temporaria was laid on its back, its breast cut open and the heart exposed. Into the lymph sac of its thigh, the same quantity of this drug was injected and its effect on the heart observed.

The contraction and dilatation gradually tended to augment and after $\frac{1}{2}$ – $\frac{3}{4}$ hour, the contraction obviously increased in intensity, while this was not the case with the dilatation. After the lapse of several hours the frequency of the heart beats gradually decreased, but hardly came to a standstill. No great difference was noticed in the course of the process even though the quantity of the drug was increased.

(iii) The addition of 0.1 mgrm. of the drug to the perfusion fluid of Straub's frog heart preparation immediately gave rise to pronounced augmentation of both the contraction and dilatation and a slight increase of the pulse frequency. This state continued for a long time, and then the amplitude very slowly decreased. Fig. 3 shows an example of those experiments. If the amount of the drug administered was increased to 0.2 mgrm., after a temporary augmentation of the contraction and dilatation, the dilatation became imperfect and in from ten to twenty minutes the heart came to a systolic standstill (Fig. 4). Usually, the number of the heart beats made a little increase, but sometimes in the course of poisoning partial auriculo-ventricular block

Fig. 3. Excised heart of *temporaria*.
(1) 0.1 mgrm. "A" substance.
(2) After 30 minutes. Time marks in 15 minutes.

and peristalsis appeared. When the drug was washed away at the moment of standstill, the heart beats recommenced slowly and the former state was recovered after a while. When the quantity of the drug was doubled, the contraction became immediately vigorous, while the dilatation was weakened and after a few minutes the standstill came.

(iv) In a Trendelenburg's thigh preparation of *rana temporaria* administration of 0.25 c.c. of 5000 fold solution of the poison was followed by no change in the amount of the outflow, but when 2000 fold solution was added a slight decrease in it occurred, while with 1000 fold solution it showed a distinct decrease (Fig. 5).

These experiments indicate that "A" substance acts on the heart and the blood vessels. Like the action of digitalis bodies, a small dose

Fig. 4. Excised heart of *temporaria*. 1.2 mgrms. "A" substance. Time marks in 15 seconds.

augments the contraction and dilatation of the ventricle, while in large dosage it inhibits dilatation and causes systolic standstill. On the blood vessels, it has the same contracting action as digitalis. The cardio-tonic action of the *kobu* decoction is presumably attributed to the presence of this substance in it.

(2) *The action of "B" substance.*

A water solution of this substance neutralized with sodium carbonate was always used.

(i) Injection of this solution into the abdominal lymph sac of the frog in a dose of 0.5 c.c. 5% solution per 10 grms. body weight slowly abolished the spontaneous and reflex movements, which gradually reappeared and were restored in 24 hours to the normal state. But when 10% solution was used, the symptoms made a rapid advance and were followed by death. The cause of these motor disturbance is no doubt the paralysis of the central nervous system.

(ii) When the heart of *rana temporaria* was exposed and the lethal dose of the drug injected into the lymph sac of its thigh, the heart beats became gradually slower, and after about three hours were followed by standstill. Application of a drop of atropine did not revive the beating, nor did mechanical stimulation.

(iii) The excised heart of a *rana temporaria* perfused with Straub's method showed on administration of a small amount of the poison a reduction in the amplitude of the ventricle beats,

Fig. 5. Blood vessel of temporaria (*Trendelenburg*). 0.25 c.c. (I)
1:5,000 (II) 1:2,000 (III) 1:1,000 solution of "A" substance.

which was due partly to the decrease of the dilatation, but mainly to the decrease of the contraction. When the quantity of the drug was increased to 1 c.c., a remarkable decrease of the amplitude was caused and in about ten minutes the heart came to a standstill in diastole. As a rule the number of the heart beats did not change, though it rarely decreased (Fig. 6).

Conclusions are drawn from these experiments, that the "B" substance has (1) a paralysing action on the central nervous system; the

Fig. 6. Excised heart of *temporaria*. Time marks in 5 seconds.
(1) 1 mgrm. "B" substance. (2) After 1 minute.

paralysis of movements caused by the *kobu* decoction is due mainly to the alcohol-insoluble component, but partly to the presence of this substance; (2) it acts on the heart, paralysing the heart muscle, decreasing the amplitude and finally causing its standstill in diastole, the cause of death due to "B" substance being brought about by this paralysing action. The reason, why in spite of the fact that a small quantity of *kobu* decoction has a cardio-tonic action, a large amount causes the diastolic standstill, may be found in the presence of this substance in the decoction.

(3) *Mannit*. Subcutaneous injection of the water solution of mannit into the *rana temporaria* in a dosage of 0.1 c.c. per 10 grms. body weight gave rise to no general symptom and no perceptible change in the heart beat. Even though 0.3 c.c. of the isotonic mannit solution was applied to the excised heart, no recognizable change occurred. But when 0.5 c.c. or more used, the contraction and dilatation, especially the contraction of the ventricle, were augmented and the amplitude increased. The amplitude tended to become smaller after a little while it did not return to the breadth before the drug was applied. This state continued for several hours (Fig. 7).

From this experiment it seems that mannit has the action of strengthening the contracting power of the excised heart. It might have the same action on the heart *in situ*, but, when subcutaneously injected, the concentration of the drug in the blood will be insufficient to cause the same effect.

Fig. 7. Excised heart of *temporaria*. Time marks in 5 seconds.
 (1) 0.5 c.c. isotonic solution of mannit. (2) After 30 minutes.
 (3) After 2 hours.

If, when on administration of 0.1 mgrm. or a quantity of "A" substance which does not bring the standstill within one hour, the contraction and dilatation of an excised heart are augmented, there is added 0.5 c.c. of the isotonic mannit solution, the contraction becomes stronger, but the dilatation remarkably decreases and finally the heart comes to standstill in systole. This state exactly corresponds to that when a large quantity of "A" substance is used (Fig. 8).

Fig. 8. Excised heart of *temporaria*. 0.1 grm. "A" substance, later, 0.5 c.c. isotonic solution of mannit. Time marks in 15 seconds.

Thus, we see that mannit potentiates the action of "A" substance. The cardio-tonic action of *kobu* decoction is apparently due mainly to the presence of "A" substance, but to some extent to this potentiating action of mannit.

(III) Summary.

The result of this series of experiments is summarised as follows : *Kobu* paralyses the central nervous system of the frogs, augments the heart beat and contracts the blood vessels. The paralysis of the central nervous system is due to the water soluble component of the active principle of *kobu*, while the cardio-tonic and the vaso-constrictive action are induced by the ether soluble component. The cardio-tonic action is weakened by the water soluble component and strengthened by mannit.

ACTION ON WARM-BLOODED ANIMALS.

The experiment in this series were all performed on rabbits. Since the active components above described are not fitted for experimenting on warm-blooded animals, the *kobu* decoction only was used in the present experiments.

(I) General symptoms.

100 grms. of dried and crushed *kobu* were heated in ten fold water on a water bath for several hours, filtered, the filtrate neutralized with sodium carbonate and reduced to 100 c.c. by heating on a water bath. When this was introduced into the stomach of a rabbit weighing 2 kilos, the ear and nose slowly became cyanotic and often the respiration was accelerated. These symptoms became more marked after forty minutes; the animal lay prostrate, was distressed, moved now and then voluntarily. After a few minutes in this state, it suddenly jumped up and fell down sideways; dyspnoea and weak heart beat. After repeated convulsions the respiration finally ceased, thereby no pulsation of the heart was discernible. When 50 grms. *kobu* was given to an animal weighing 2 kilos, no perceptible symptom was observed, but it accelerated urination. Intravenous administration of the decoction caused even in a minute dose those symptoms. The lethal dose is from 1.0 to 2.0 grms. of *kobu* per 1 kilo body weight.

Thus, the *kobu* decoction paralyses the respiration and the heart and finally causes death. Whether the immediate cause of death is its action on the respiration or on the heart is to be determined by the following experiments.

(II) Action on the respiratory movements and the blood pressure.

Of a rabbit completely anaesthetised with urethane, one nasal cavity was connected with a tambour and the carotid artery on one side

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Fig. 9. Rabbits ♂ 1.85 kilos. Respiration and blood pressure. Time marks in 5 seconds. Intravenous injection of 30 c.c. 10 % *kobu* decoction. (1) Before injection. (2) After injection.

with a manometer, in order to record the respiratory movements and the blood pressure. When 2 c.c. of 10 % *kobu* decoction per kilo body weight was slowly injected into the jugular vein, there was no change in the respiratory movement, while the blood pressure was pronouncedly influenced. Generally the blood pressure showed after a preliminary decline a gradual rise lasting for some time, then it was lowered again and after a long period restored to the normal height. The preliminary fall of the blood pressure was almost imperceptible, if the injection took place slowly. Repeated administration gave rise to further elevation of the blood pressure; but when a large quantity (10–20 c.c.) was applied, it fell sometimes to the zero line. When the blood pressure was markedly lowered, the respiratory movement became quick and lively, but finally it became slow and weak until it ceased after a few minutes. When a very large dose was given to the animal at one time, it gave rise to immediate fall of the blood pressure without preceding elevation and caused the death of the animal. Fig. 9 shows the relation between the cessation of respiration and lowering of blood pressure in the case when a large dose was injected at one time.

Thus, a large quantity of *kobu* decoction induces the respiratory and circulatory disturbance, the latter being the direct cause of death. The change of the respiratory movement is probably attributed to the stimulation of the respiratory centre due to the fall of blood pressure. Hence an investigation in detail on the action of the decoction upon the circulatory system has been made as follows.

(III) Action on circulation.

It has already been stated that intravenous injection of the *kobu* decoction in a small quantity raises in rabbits the blood pressure after a preliminary slight fall, while in a large dose it is lowered until death follows. Fig. 10 shows the influence on the blood pressure. In order to elucidate this change in the blood pressure, the action on the heart was first examined.

(1) *Action on the heart.* A rabbit was completely anaesthetised with urethane, the trachea cut open and connected with the artificial respiration apparatus, the breast opened, the heart exposed, and its left ventricle was connected with Cushny's myocardiographic apparatus, by means of which pulsation was recorded. When 10 c.c. 10% *kobu* decoction was slowly injected into its vein, there occurred in a little while an augmentation of the contraction and dilatation which was

Fig. 10. Rabbit ♂ 2 kilos. Blood pressure. Time marks in 5 seconds.
 (1) Intravenous injection of 10 c.c. 10 % *kobu* decoction. (2) After 10 minutes. (3) After 15 minutes. (4) After 17 minutes. (5) After 20 minutes.

gradually accelerated until the amplitude sometimes became three times as large as the original one. But later the amplitude gradually become smaller and the heart came to a standstill, as in the case of the frog (Fig. 11).

Thus, evidence is put forward that the *kobu* decoction has the action of augmenting the contraction and dilatation of the heart from the beginning. Probably this is due to the presence of "A" substance in the *kobu* decoction. The reason why the *kobu* decoction causes the rise of blood pressure may be because of this cardio-tonic action. But at the beginning in spite of the strengthened heart-beat, there is a temporary fall of blood pressure. Is this not because the drug has also the action of dilatating the blood vessel? In order to answer this question, the next experiment was performed.

(2) *The influence of kobu on the blood pressure and the organ volume.* Of a urethanized rabbit the carotid of one side was connected with the manometer, and a part of the small intestine was placed in an oncometer, so that the blood pressure and the volume of the small intestine might be simultaneously recorded. When the *kobu* decoction was injected into the vein, the blood pressure fell for a time and then

Fig. 11. Rabbit ♂ 2.1 kilos. Heart beats. Time marks in 4 seconds. Intravenous injection of 10 c.c. 10% *kobu* decoction. (1) Before injection. (2) 10 minutes after injection. (3) 18 minutes after injection. (4) 25 minutes after injection. (5) 30 minutes after injection. (6) 40 minutes after injection.

gradually rose. The volume of the small intestine increased with the fall of blood pressure, and when the blood pressure rose again, the volume instead of decreasing made a still further increase, later showing a tendency to change parallel to the blood pressure (Fig. 12).

It is now obvious that the *kobu* decoction has an action, besides magnifying the work of the heart, to dilate the blood vessels of organs. The temporary fall of the blood pressure immediately following the injection of the drug is due to the overcoming of vaso-dilatative action by the cardio-tonic effect, while the later rise of the blood pressure is caused by the superiority of the latter over the former. Whether its action on the blood vessel is central or peripheral is not clear. But inferring from the experiments on the blood vessels of the frog, it seems very probably that it is central. It is an open question whether this action is due to "A" substance or to some other components of the *kobu* decoction.

Fig. 12. Rabbit ♂ 1.95 kilos. *a.* Volume of intestine. *b.* Blood pressure. *c.* Time marks in 5 seconds. Intravenous injection of 5 c.c. 10 % *kobu* decoction. (1) Before injection. (2) 2 minutes after injection. (3) 5 minutes after injection. (4) 10 minutes after injection.

(IV) Action on the formation of urine.

As stated in the observation of general symptoms, a small quantity of the *kobu* decoction frequently gives rise to urination. It might be that the cardio-tonic action of the decoction induces the rise of blood pressure, which is followed by diuresis, or that, as in the case of the small intestine, the blood vessels of the kidneys are dilatated and thus facilitating the renal circulation accelerate the urine secretion. In order to determine this problem the variation of urine secretion together with the change in the blood pressure and the volume of the kidney was observed. A rabbit was anaesthetised and fixed on its back, the carotid artery connected with a manometer and the kidney placed in an oncometer. The bladder was cut open and a collecting funnel inserted. Drops of urine flowing from this collector were received into the drop recorder which traces the quantity of urine on a smoked paper. Now after the blood pressure, the volume of the kidney and the quantity of urine became constant, 2 c.c. 10 % *kobu* decoction was slowly injected into the vein. The blood pressure, after a temporary slight fall, was

slowly raised, while the volume of the kidney irrespective to that was gradually enlarged. The quantity of urine increased with the swell of the volume of the kidney, and was further augmented with the rise of blood pressure. Fig. 13 shows an example of these experiments.

Fig. 13 Rabbit ♂ 2 kilos. Intravenous injection of 2 c.c. 10 % *kobu* decoction. a. Volume of kidney. b. Blood pressure. c. Urine secretion (drops). d. Time marks in 5 seconds.

It may be concluded from this experiment that the *kobu* decoction accelerates the circulation in the kidney in a double way: by dilating the renal blood vessels, and by raising the blood pressure. Again, the *kobu* decoction contains a large amount of mannit which as is already known has a diuretic action. All these conditions work in favour of the diuresis.

V. Summary.

The *kobu* decoction has both a cardio-tonic and a vasodilatative action. In its action at first the latter overcomes the former and brings forth a slight fall of the blood pressure, while a little later just the reverse is the case, the blood pressure being raised. The urine excretion is augmented owing to its action dilatating the renal blood vessel, which is aided by the diuretic action of mannit contained in

it. A large dose of the decoction is followed by a disturbance of the heart beat, together with the dilatation of blood vessels, which may induce the fall of blood pressure and consequent death.

CONCLUSIONS.

Kobu has a cardio-tonic and vaso-dilatative action. Quick administration into the circulation of a small quantity causes a slight preliminary fall of the blood pressure followed by a subsequent rise. But when it is administered very slowly, there is no fall, but only a rise of blood pressure. The dilation of blood vessel, and the consequent decrease of peripheral resistance together with the simultaneous rise of blood pressure accelerates the circulation of blood in various organs. The popular use of this plant as a cardio-tonic has some pharmacological grounds for its use.

Kobu induces diuresis. This action originates partly from the rise of blood pressure due to the cardio-tonic action and the dilatation of the blood vessel of the kidneys, and partly from the diuretic action of mannit contained in this plant.

Thus, though *kobu* contains some heart poison, it is daily used as a common food stuff without producing any known instance of intoxication. This is chiefly due to the largeness of its lethal dose, this being, given internally to the rabbit, about 50 grms. per kilo of body weight. That there is a marked difference in the lethal dose between the stomachal and intravenous administration suggests that it is not easily absorbed from the intestines, although it has a strong cardio-tonic action.

It is the desire of the author to succeed in separating the active principle of this plant and to be able to give a further report on the subject.

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