STUDIES ON AN ANTIDIURETIC SUBSTANCE IN THE BILE

REPORT I. DEMONSTRATION OF AN ANTIDIURETIC SUBSTANCE IN THE BILE

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The amount of outflow of the duodenal juice (bile) obtained by a four hours' drainage was compared with that of the urine obtained during the same period in seven normal persons. The mean bile volume (70 ml. per hour) and the mean urine volume (55ml. per hour) made up a total fluid loss of 125 ml. per hour, or about 3 liters a day (Table 1). It thus indicated that, with respect to body fluid balance, there was no decrease of the urine volume compensating for the biliary fluid loss. In view of the regulatory mechanism with which the organisms are usually endowed, such a phenomenon seems to be unreasonable. A loss of some antidiuretic substances through the bile would possibly explain this. Therefore attempts have been made to seek an antidiuretic substance in the bile, which may be of a vasopressin-like nature.

Table 1 Comparison of the Serum, Bile and Urine in Normal Persons

		Volume	Cl	Na	K	NPN	UN
Concentration (in reference to the concentration in serum)	Serum	—	1	1	1	1	1
	Bile	—	0.9	0.9	1.3	3.5	1
	Urine	—	1.8	1.2	7.0	2.5	40
Excretion in one hour (Average)	Bile	70ml	280mg	220mg	20mg	50mg	10mg
	Urine	55ml	350mg	200mg	60mg	300mg	220mg

Urine and bile (duodenal juice) were simultaneously obtained during 4 hours in 7 persons.

METHOD

1. Assay of antidiuretic activity

Assay method for antidiuretic activity was essentially the same as the technique

described by Birnie[1]. A group of three rats weighing approximately 150 gm were fasted but allowed to drink water in the morning of assay. Two doses of 5 ml. warm water (**A** ml.) were administered by a stomach tube at hourly interval to animals which were placed in a metabolic cage and the urine excreted in the ensuing 2 hours was measured (**B** ml.). At the time of the third dose of water, the animals were injected intraperitoneally with 0.5 ml. of the test material. The urine volume was measured for 90 minutes thereafter (**C** ml.). The ADS-index as an antidiuretic activity was calculated by the following formula.

$$\text{ADS-index} = \frac{\mathbf{C}}{3\mathbf{A}-\mathbf{B}} \times 100$$

Any group which, during the hydration period, had a urine volume below 10 ml. or above 20 ml. was discarded.

The urinary chloride concentration was measured according to the method described by Schales and Schales[2], and the Cl-index was calculated by the following formula.

$$\text{Cl-index} = \frac{\text{Cl concentration of urine in 90 minutes after the injection of test material}}{\text{Cl concentration of urine in 120 minutes before the injection of test material}}$$

2. Extraction of antidiuretic substance (treatment of bile)

Since a vasopressin-like substance was suspeted to be present in bile, quantitative recovery of Pitressin (a vasopressin derivative, Parke Davis Co.) was attempted with the following extraction technique.

Fresh human bile obtained by duodenal drainage was diluted with an equal volume of distilled water and its pH brought to 5 with hydrochloric acid. To a 100 ml. portion thereof was added 32 ml. of 2N potassium ferrocyanide and 30 ml. of 2N zinc sulphate under vigorous stirring. The mixture was centrifuged and the precipitate was extracted three times with 5 volumes of 1% ammonia in 80% ethanol. The extract (ethanol fraction) was concentrated to dryness in vacuo at 50°C. The residue was extracted with 5 ml. of 0.25% acetic acid. The acetic acid extract was then filtered and the filtrate was extracted with 50 ml. of isobutanol. The extract (isobutanol fraction) was concentrated to dryness in vacuo. The remaining dry material was subjected three times to the same isobutanol extraction to remove resinoid material which was still contained in the last dry material, could be eliminated through filtration after suspending it in normal saline, since it was almost insoluble in it. Experimental animals tolerated an intraperitoneal or intravenous injection of a solution of this material.

RESULTS

1. Recovery of Pitressin

Two hundred milliunits of Pitressin diluted with 20 ml. of water was treated with the extraction technique mentioned above. Then the extract was divided into 3 equal portions and injected intraperitoneally to 3 rats for the estimation of the ADS-index. The ADS-index thus obtained was nearly the same as that obtained in a group of rats receiving an intraperitoneal injection of 200/3 mU of Pitressin (Fig. 1). By this technique, the recovery of Pitressin from the water solution seemed to be fairly reliable. The ADS-index obtained in a blind experiment was the same as that of the control (Fig. 1). The Cl-index was decreased by this extraction procedure, and the activity of Pitressin to elevate urinary chloride concentration was decreased (Fig. 2).

The ADS-index, assayed on the mixture of 20/3 ml. of human C-bile and 200/3 mU of Pitressin ranged from 0.5 to 1.0, indicating higher values than that of bile without Pitressin. The ADS-index assayed on 20/3 ml. of bile without addition of

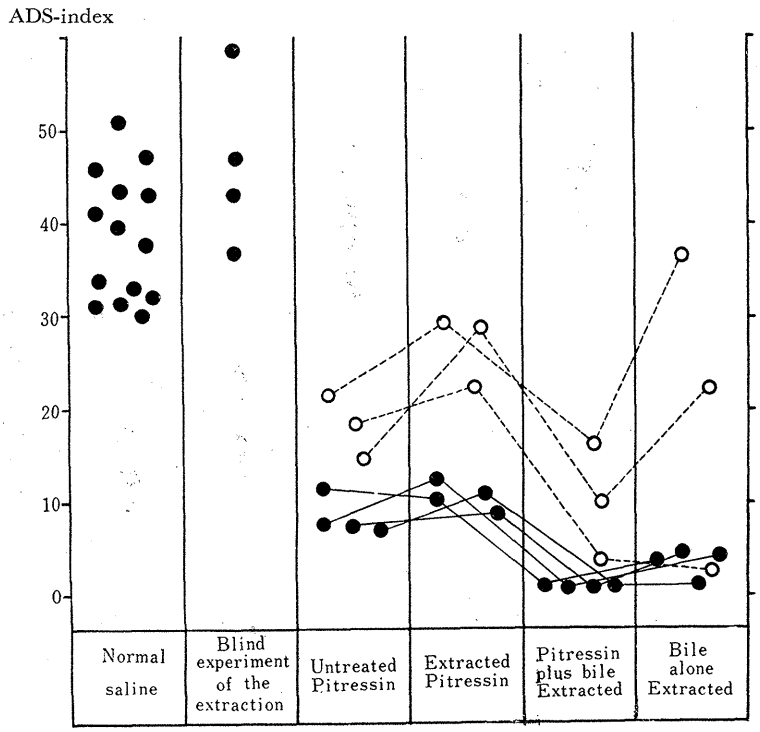

Fig. 1 Recovery of Pitressin (ADS-index)
Simultaneous experiments using Pitressin from the same ampules were connected with line.
Solid line group: Pitressin 200/3 mU, and/or bile 20/3 ml for each rat.
Broken line group: Pitressin 150/3 mU, and/or bile 10/3 ml for each rat.

Pitressin ranged from 0.5 to 4.0 (normal ADS-index: 38.0±6.8) (Fig. 1). The result suggests that the bile itself has an antidiuretic activity. The antidiuretic activity of the bile made the quantitative recovery of Pitressin from the bile unsuccessful. A nearly perfect recovery of Pitressin was obtained from a half volume of bile in the cases in whom the biliary antidiuretic activity was low.

Pitressin lost its original urinary chloride concentrating activity during the extraction from the bile with which it was mixed (Fig. 2).

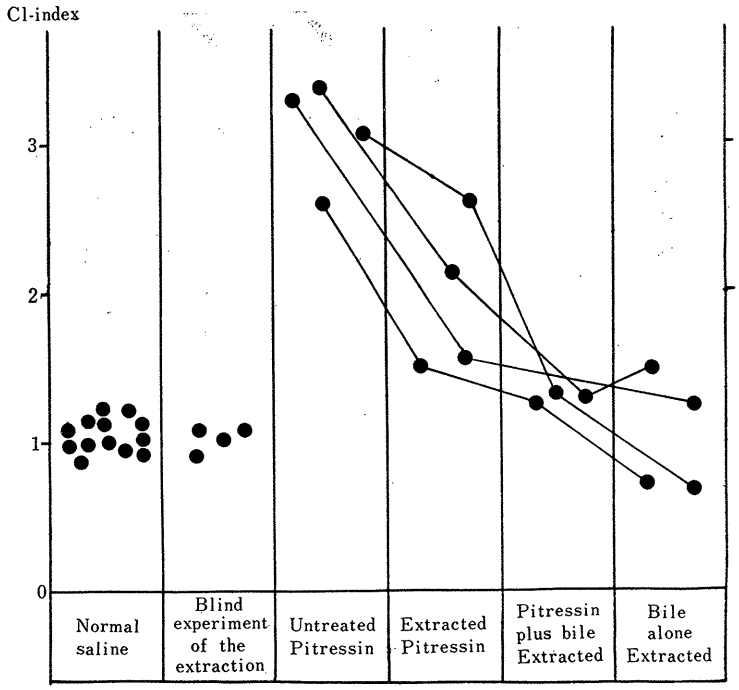

Fig. 2 Recovery of Pitressin (Cl-index)
Simultaneous experiments using Pitressin from the same ampules were connected with line.

2. Antidiuretic substance in human bile

The extract from 20 ml. of human C-bile was divided into 3 equal portions and injected intraperitoneally into rats. The ADS-index thus obtained was lower than that of controls in 9 cases excepting one, thus confirming the existence of an antidiuretic substance in the bile (biliary ADS) (Fig. 3).

3. Demonstration of an antidiuretic substance in the bile by a modified technique

Human C-bile was brought to pH 3 with hydrochloric acid and concentrated to

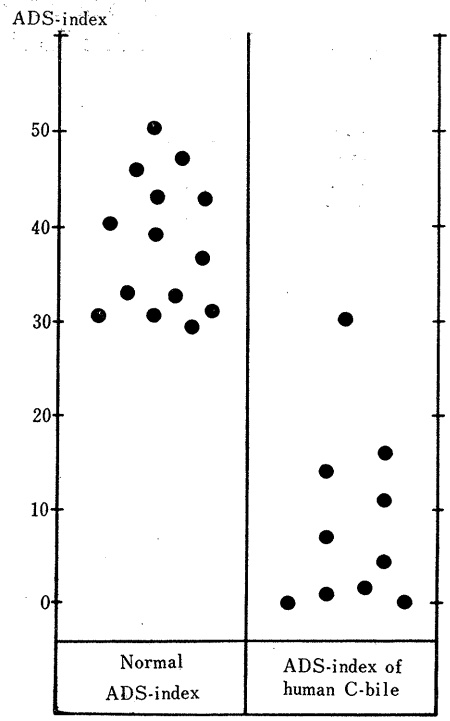

Fig. 3 Antidiuretic activity of human C-bile

dryness in vacuo. Rotation flash evaporater was conveniently employed in this procedure. The material remaining was subjected to each extraction step with ammonia-ethanol, glacial acid and isobutanol in due order, without precipitation with zinc sulphate and potassium ferrocyanide.

The ADS-index of the material thus obtained ranged from 1.6 to 7.7, an antidiuretic substance being obtained from the bile by this modified procedure also (Table 2). The Cl-index of the material ranged from 1.10 to 2.45, somewhat higher than in the normal.

Table 2 Antidiuretic Activity of the Human Bile (Modified Extraction Procedure)

No.	ADS-index	Cl-index
1	7.7	1.10
2	1.6	2.09
3	6.1	5.24

4. Examination of antidiuretic activities in known biliary components

Intraperitoneal injection of 3.33 mg of bilirubin, sodium cholate, sodium desoxy-

cholate, sodium taurocholate or glucodesoxycholate in 0.5 ml. water revealed no antidiuretic activity (Table 3). The results suggest that the biliary ADS is a hitherto unknown substance.

Table 3 Antidiuretic Activity of Some Biliary Components

Materials	No.	ADS-index	Cl-index
Normal saline	1	30.5	1.00
	2	31.6	0.81
	3	30.9	1.10
Bilirubin	1	28.9	0.86
	2	30.6	0.82
	3	36.8	1.00
Sodium cholate	1	35.4	1.00
	2	48.0	0.83
	3	48.2	1.00
Sodium desoxycholate	1	30.6	1.00
	2	34.5	0.89
	3	30.1	0.85
Sodium taurocholate	1	28.7	1.11
	2	37.1	0.92
	3	57.3	1.11
Sodium glucodesoxycholate	1	45.5	1.00
	2	62.7	1.00
	3	54.1	0.88

DISCUSSION

Pitressin (a vasopressin derivative) was nearly quantitatively extracted from a water solution in the following manner; first, precipitation with zinc sulphate and potassium ferrocyanide, and second, extraction of the precipitate with ethanol, glacial acid and isobutanol in its order. With application of this extraction procedure on fresh human bile, an antidiuretic substance was demonstrated in the bile.

The antidiuretic substance in the bile (biliary ADS) was different from Pitressin itself in its inability to elevate urinary chloride concentration. However, it can not be concluded that the biliary ADS is very different from Pitressin, because of the fact that Pitressin loses the urinary chloride concentrating ability after receiving the same extraction procedure. The elucidation of this problem awaits further studies.

There was no compensatory decrease of the urine volume corresponding to the large biliary fluid loss in patients in whom continuous duodenal drainage was carried out. It is presumed that such a phenomenon resulted from imbalance of the body fluid, which was brought about by the loss of blood antidiuretic substance in the form of biliary antidiuretic substance.

We had experience with an anuric patient (100 ml. of urine per day) in whom a

remarkable diuresis (2700 ml.) was obtained on the next day following duodenal drainage which removed 3000 ml. in 24 hours.[3] Such a phenomenon may have resulted from the loss of biliary ADS.

CONCLUSION

An antidiuretic substance was demonstrated in human bile. It could be extracted by a technique which is primarily used for extracting vasopressin, a pituitary posterior hormone. The biliary antidiuretic substance was different from bilirubin, sodium cholate, solium desoxycholate, sodium taurocholate, or sodium glucodesoxycholate.

REFERENCES

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