ENDOCRINOLOGICAL STUDY OF ACUTE ILEUS

GEN'ICHI SHIRAKAWA*

Matsukura Surgery Clinic, Nippon Medical School

THIRTEEN FIGURES

It is very important to study the acute ileus from the endocrinological standpoint. As the acute ileus is considered as a type of "stress", the endocrinological study must be started from the role of hypophysis-adrenocortex-system. And the adrenocortical function is suggested by the 17-ketosteroid (17-K. S.), 17-hydroxycorticoid (17-H. Y.), chemocorticoid (C. H.) in urine and also uric acid, creatinine ratio and the eosinophil count after the ACTH and adrenalin injection. The role of adrenocortex in acute ileus is studied biochemically.

METHOD OF ESTIMATION

A) It is inevitable to estimate total 17-H. Y. and C. H. for the evaluation of the adrenocortical function. The method to estimate the total 17-H. Y. in urine was devised by G. W. Thorn, *et al.* Its estimation in our clinic is carried out by the Ichikawa-Waku-Ishimoto's method, which is a modified Thorn's method.

Materials [I] n-Butanol (purified)

Purified Butanol on the market in Japan still contains considerable amounts of chromogens which must be eliminated by the following steps, details of which are as follows: (cf. *Endocrinologia Japonica* 1:81, 1954.)

(1) To shake butanol with 0.2 vol. 10% NaHSO₄ solution for 5 mins. The 10% NaHSO₄ solution is made beforehand. Then to manage the butanol 400 cc. in separating funnel.

(2) To wash it with 0.1 vol. distilled water.

(3) To add 1 ml. of conc. H₂SO₄ containing 50 mg. of phenylhydrazine hydrochloride, into 500 cc. butanol, and heat the whole mixture at 60~65°C for about 30 mins. on the water-bath.

(4) After cooling off, to wash twice with 0.1 vol. distilled water.

(5) To wash with 0.1 vol. NaOH (1 N) for 3 mins.

(6) To wash twice with 0.1 vol. distilled water.

(7) To dehydrate with anhydrous Na₂SO₄ for one night.

(8) To distill the butanol after the dehydration.

[II] Phenylhydrazine hydrochloride (purified)

It is necessary to purify and recrystallize the phenylhydrazine hydrochloride on the market. Add 20 cc. alcohol (80%) to 5 g. of phenylhydrazine hydrochloride

and dissolve at the temperature over 80°C until it becomes saturated. Put into this, 2 mg. of animal charcoal powder and boil with burner. Filtrate it using a funnel (dia.=3 inch) and filtrating paper into a vessel to cool. Then exchange for 3G₃ glass filter, pour pure alcohol into it, filtrate it with sucking, and the rest of the alcohol is abolished. Make a heap laying sheets of filtrating paper and arrange the pieces of crystal on each sheet. Put this whole heap into the desiccator for 2 nights, then the dried crystal pieces are scraped off together and kept in a dry place. The above process needs a fair technique.

(3) Dil. H₂SO₄ (620 cc. conc. H₂SO₄+water=1 L. of dil. H₂SO₄ totally)

(4) Phenylhydrazine-H₂SO₄ reagent

Dissolve the 65 mg reagent into 100 cc. of (3). This solution is used after fixing for one week at 0~4°C. This reagent must be renewed every one week.

(5) HCl-Semicarbazid reagent

(6) Adrenocortex hormone standard solution

Butanol+cortisone acetate 5~50 γ/1 cc.

Procedure: Preserve the 24-hour urine at the room temperature. Addition of small amount of n-butanol will do. Take up 15 cc urine and regulate pH of it to 1.0 by dropping H₂SO₄ (Test paper and cresol should be used). Then add 0.5 vol. purified butanol and stir up in a separating funnel for at least 3 mins. and extract from it twice in succession. The emulsion is easy to be separated by centrifugal force using centrifuging tube of 50 cc. in volume.

ROUTINE COLORIMETRY

A-test tube; Butanol extract 1.0 cc.+0.65 mg/cc. Phenylhydrazine-H₂SO₄ reagent 4 cc.

B-test tube; Butanol extract 1.0 cc.+HCl. Semicarbazide 15 mg. Heat the mixture in the bath at 100°C. for 10 mins. After cooling, add 4 ml. dil. H₂SO₄ reagent.

Mix A and B perfectly, heat at 60°C exactly for 20 mins.; after that cool for 5 mins. in the cold water bath. Adjust the blank to 0 with distilled water and measure the absorbance=log T with 410 mμ filter. For the purpose of blind test 15 ml. distilled water is used instead of urine. The same treatment is applied. Then regulate it to pH 1.0, wash twice with 0.5 vol. butanol, then it is applied to colorimetry. This is divided into two tubes, namely these two are the "a" and "b" tube used for blind test. When pH is adjusted to 1.0 and 5.0~6.0 by adding distilled water, comparing "a" and "b", we can easily find that the tube of pH 5.0~6.0 shows a higher intensity of colour of phenyl base than that of pH 1.0. So pH should be regulated to 1.0 in the blind test (Table I). Often during

Table I. pH of the distilled water

pH	Casenumber	Absorbance		a-b
		bea tube	b tu	
1.0	1	0.011	0.002	0.009
	2	0.013	0.002	0.011
5~6.0	3	0.009	0.004	0.005
	4	0.093	0.011	0.002

the colorimetry there appears some cloud-like floating substance. We must begin the test after entire precipitation of it.

Table II. Effects of heat to cortisone ($\gamma/40\text{cc.}$)

	80~85°C	90~95°C	95°C
1	184	216	216
2	76	104	88
3	121	146	136
4	134	168	152
5	256	284	262
6	136	176	168

$A-B=\alpha$ (regular tesse), $a-b=\beta$ (blind test), $\alpha-\beta=N$ (asked value)

Change of Calibration Curve :

In the colorimetry the author used 410 $m\mu$ filter. Here the appointed amount of semicarbazide hydrochloride is not always 15 mg., but should be so arranged, *i. s.* 15 mg. in autumn and winter, 20 mg. in summer. $A-B=+0.005\sim-0.015$

Fig. 1.

B) The estimation of chemocorticoids in urine is carried out by Nakao-Aizawa's method. The effect of heat to cortisone is studied and found that it is

stable against relatively lower temperature but destroyed by higher temperature. The optimal temperature is found to be 90~95°C.

C) 17-K.S. is measured by Miyake's method, the modified Dreker's method. For the purification of alcohol 1.5 g. of AgNO_3 crystal is dissolved in 3 cc. distilled water and added to 1 L. of alcohol (on the market). After the dehydration by CuSO_4 , it is distilled. The first and last 10% of effluent should be discarded in order to avoid aldehyde and ketone.

Removal of pigments from urine by animal-charcoal, recovery rate of dehydroisoandrosterone and methyltestosterone propionate become satisfactory (Table III).

Table III. The effect of pigments in the measurement of 17-K.S. (mg. %)

	Pigment		Correction	
	still contained	not contained	D. I. S.	M. T. P.
No. 3	0.9	0.8	0.82	0.81
No. 14	0.75	0.7	0.71	0.69
No. 25	0.85	0.8	0.8	0.78
No. 36	1.10	0.9	0.92	0.92

According to Miyake's method, conc. HCl (6 cc.) was added to 20 cc. of urine specimen and heated, then average 23.5 cc. filtrate was obtained after cooling. The sample (10 cc.) was taken up from this filtrate in order to measure 17-K. S. Therefore, the following calculation must be done after measurement (Fig. 2.).

Fig. 2. The calculation of 17-K.S.

Measured value $\times 23.5/7.7 \times K/20 = 17\text{-K.S. value asked (mg./day)}$.

The loss of urine by heating must be considered to obtain the exact results. The value of this formula will be shown clearly in the following clinical example (Table IV).

E.K., 38 years old woman, strangulations-ileus patient.

Table IV.

	Just before operation	Just after operation	The day after operation	The 4th post-opera- tive day	The 7th post-opera- tive day	The 10th post-opera- tive day	2 weeks after leav- ing hospital
Estimated value	5.25	10.4	18.52	8.85	9.9	11.65	19.42
Corrected value	6.75	12.2	21.77	10.41	11.64	13.70	22.84
Corrected rate	0.160	0.173	0.175	0.176	0.175	0.176	0.176

D) Creatinine was measured by Jaffe's method.

E) Uric acid was measured by cyanide urea method.

F) Eosinophile count. (1) Adrenalin Test: 4 hours after the subcutaneous 0.3 mg. adrenalin-HCl injection the decrease of eosinophilic cells in blood stream was measured. Eozie Y (made in Germany) is used. Hinkelman's reagent is preserved in refrigerator. (2) ACTH test: The same eosinophil count was carried out after intramuscular injection of 25 mg. ACTH (Armour). Uric acid-creatinine ratio is calculated at the same time.

CLINICAL RESULTS

a) The average results in 28 normal adult men and women are follows:

Total 17-H. Y. in.....men 5.0~13.0 mg./day; women 4.7~12.0 mg./day.

C. H. inmen 4.7~8.4; women 3.3~7.2.

17-K. S. inmen 9.0~21.0; women 6.5~14.0.

Uric acid in.....men 0.3~0.4; women 0.3~0.4.

Creatinine inmen 0.4~0.5; women 0.3~0.4.

b) The clinical results in acute ileus.

Total 17-H. Y. (Fig. 2, 3 and 4), C. H. and 17-K. S. in acute ileus are diminished below normal: 17-H. Y. 0.75~3.8 mg./day, C. H. 0.5~3.5 mg./day, 17-K. S. 1.5~5.5 mg./day.

(1) Total 17-H. Y., C. H. and 17-K. S. increased for 2 days after operation then began to decrease for following 2 days, and gradually returned to normal in well recovered patient (Fig. 6).

(2) Similar course of endocrinological changes are observed in well recovered cases of acute generalized peritonitis. Little changes of these adrenocortical hormone in urine are observed in severe cases. (Fig. 7).

A case of complicated acute generalized peritonitis strangulations-ileus is described as follows;

Total 17-H. Y., C. H. and 17-K. S. remarkably increased for 2 days after operation of peritonitis then gradually decreased for 4 days on account of acute ileus. And after the surgical operation for ileus it increased once more for 2 days, and followed the gradual fall till the 7th day and it returned to normal (Fig. 8).

(3) Uric acid in acute generalized peritonitis is 0.104 mg./day, creatinine 0.104~0.117 mg./day. Uric acid-creatinines ratio increased more than 50 % for 2 days after operation and then began to decrease. In cases complicated with acute ileus this ratio decreased about 30%. After the operation of ileus, it increased again 30%. After the tendency of slight decrease, it began to increase again from the 7th day after operation of ileus (Fig. 9).

Fig. 3.

Fig. 4.

Fig. 5.

The curve of total 17-H.Y.,C.H. in urine & 17-K.S. in urine in Ileus

Fig. 6.

The curve of total 17-H.Y. in acute generalized Peritonitis

Fig. 7.

ANIMAL EXPERIMENTS

Artificial strangulations-ileus at the upper part of the small intestine of dogs were studied. This artificial ileus was continued for $1\frac{1}{2}$ ~2 hours. Total 17-H.Y., C.H., 17-K.S. and pH of urine and uric acid-creatinine ratio were estimated successively.

Fig. 8.

(1) Total 17-H.Y., C.H. and 17-K.S. in urine decreased remarkably at the acute artificial ileus. After the release of ileus they began gradually to increase and then became highest at 48 hours after release, and then turned to decrease for 72 hours. This result coincides with the clinical result.

(2) The uric acid-creatinine ratio decreased 40% at 48 hours after release, then they turn to increase (Fig. 11).

In normal dog total 17-H.Y. was 0.43 mg.%, C.H. 0.3 mg.%, 17-K.S. in urine 0.8 mg.%, uric acid 18.6 mg.% and creatinine 26.2 mg.% and the average specific gravity of urine was 1.018, average pH of urine was 7.4.

(3) After the release of ileus, the urine was acidified (pH 6.2), and it recovered to normal paralleled with the recovery. Being the shock severe enough to cause death, pH of urine went to fall.

(4) The uric acid-creatinine ratio and the eosinophil counts were examined

C.H. in urine acute Peritonitis→Str. Ileus (16 year woman)

Fig. 9.

Fig. 10.

Uric acid-creatinine ratio acute peritonitis→Ileus

Fig. 11.

Fig. 12.

Fig. 13.

at ileus condition. Injection must be carried out carefully to the dog which is apt to fear it.

i) In normal dogs injected adrenalin (0.3 cc., 1:1000), uric acid-creatinine ratio increased 58% and the eosinophile count decreased 16%. In normal dogs injected, ACTH (Armour's) 25 mg. intramuscularly, uric acid-creatinine ratio increased 179%, eosinophil count decreased 75%. Reaction to adrenalin is not obvious but that to ACTH was remarkable.

ii) In dogs just after the release of ileus, ACTH was administered. Uric acid-creatinine ratio was +91% and the eosinophils in blood is none. It seems to me that in this period the reaction caused by ACTH does not appear but the reaction against stress itself. During 72 hours after artificial ileus the uric acid-creatinine ratio gradually declined, and the eosinophils cannot be found in any case. (Table 5)

Table V. Thorn's test in ileus dog (ACTH 25mg.) released from ileus

Ileus dog	Uric Acid	Eosinophils
	Creatinine	
No. 3 dog no treatment	+90	Disappeared
No. 4 released	+91	"
No. 5 after 4 hrs.	+43	-25
No. 6 " 8 "	-3	Disappeared
No. 7 " 24 "	-25	"
No. 8 " 48 "	-30	"
No. 9 " 72 "	-40	"

CONCLUSION

(1) The estimations of total 17-H.Y., C.H., 17-K.S., creatinine and uric acid were carried out clinically (9 acute ileus patients, 15 controls of other diseases) and experimentally (26 artificial ileus dogs).

(2) Total 17-H.Y., C.H. and 17-K.S. in ileus patients are remarkably decreased compared with those of normal.

(3) After the operation these hormones are increasing till the first or second postoperative day and then begin to decrease till the 4th day, and then turn to increase again from 5th day to normal in well recovered cases.

(4) The adrenocortical hormones show little changes in severe cases.

(5) The results on dogs are very suggestive, for they agree with those of clinical examinations.

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