Influence of Estrogen on Adrenocortical Function in Totally Depancreatized Dogs

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Influence of Estrogen on Adrenocortical Function in Totally Depancreatized Dogs

by

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I. INTRODUCTION

Total pancreatectomy was successfully carried out in dog by JOHANN CONREMD BRUNNER in 1682. THOMAS CAWLEY52> reported in 1788 close correlation between pancreatic impairment and diabetes mellitus accompanied by polyuria, which was followed by an observation of pathologic findings in the pancreas of diabetics by ROKITANSKY. In 1889, MEHRING & MINKOWSKI19 ascertained an occurrence of diabetes mellitus after total pancreatectomy, and various experimental studies have been carried out such as those of DRAGSTEDT2> in 1943. On the other hand, total pancreatectomy was for the first time successfully carried out in man by ROCKEY3> in 1943, and in our country the first successful case was reported by HONJO15> in 1954. Essential problems of pathophysiology after total pancreatectomy consist not only in the disappearance of pancreatic juice as exocrine secretion, but also in the disappearance of proper insulin as endocrine secretion as
well. In this respect, it differs essentially from pathophysiology of partial pancreatectomy in which secretion of proper insulin remains. It is recognized from the findings of experiment made by Nishikawa that general condition is kept better with less decrease in body weight in totally depancreatized dogs when estrogen is administered additionally to insulin, compared with administration of insulin alone. The author of the present paper produced following 4 groups of totally depancreatized dogs, i.e. group of insulin administration alone, group of estrogen administration alone and group of simultaneous administration of insulin and estrogen. In each of these groups, various adrenocortical functions were comparatively studied. Namely, urinary total 17-KS, its thin layer chromatography, urinary total 17-OHCS, its thin layer chromatography, 17-OHCS in plasma, eosinophil count in peripheral blood, response to Thorn test, proportion between adrenal weight and body weight and histological findings of the adrenocortex were investigated.

II. MATERIALS AND METHODS

Healthy adult mongrel dogs weighing around 10 kg were employed in the experiment. After feeding of approximately 4 days, preoperative examinations were carried out and blood and urine were taken. Animals were subjected to operation in the fasting state. Since the pancreas of dog is not fixed in the retroperitoneal cavity, removal of this organ can be carried out with ease.

A. Determination of Urinary Total 17-KS (Modified KANEGAWA's Method)

1. Reagents

   i) Ethyl ether of special class
   ii) H₂SO₄ of special class
   iii) 20 per cent formalin solution
   iv) 4 per cent NaOH solution
   v) 1 per cent m-dinitrobenzene-ethanol solution
   vi) 8N-KOH solution
   vii) 80 per cent ethanol solution
   viii) Standard solution of dehydroepiandrosterone (abbreviated to DEAS hereafter)

   Ten mg of DEAS was weighed on Metra balance, and dissolved in 100 cc of ethanol of special class. Stopper of the funnel was covered with foil in order to prevent an increase in titer.

2. Method of determination

   i) Ten cc of 24 hours' total urine was put in a centrifugation tube and 0.2 cc of formalin solution was added.
   ii) Hydrolysis: One cc of conc. H₂SO₄ was further added and well mixed in the tube. The tube was placed in boiling water of 100°C for 15 minutes, which was then cooled down rapidly in ice-water.
   iii) Extraction: Thirty cc of ethyl ether was added to the above mentioned urine, which was well shaken for extraction and isolated in a separation funnel.
   iv) The material was rinsed twice with 5 cc of 4 per cent NaOH solution, and
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when still stained it was rinsed further.

v) The material was rinsed 3 times with 10 cc of distilled water.

vi) After rinsed, it was desiccated with Na₂SO₄.

vii) It was furthermore desiccated under negative pressure.

viii) Standard solution of DEAS of 0.5 cc was taken precisely in a pipette, and dried material, which was desiccated in centrifugation tube under negative pressure, was regarded as standard material.

ix) ZIMMERMANN⁷) reaction: One per cent of m-dinitrobenzene-ethanol solution of 0.4 cc was added, and 0.2 cc of 8 N-KOH was added and well mixed, which was then let stand in room temperature for 30 minutes (60 minutes in winter). Then 4 cc of 80 per cent ethanol solution was added. After 5 minutes, optical absorption was determined by spectrophotometer of KOLLMANN through 460 μ, 520 μ and 580 μ.

x) Calculation: Allen’s formula for correction

\[ E = \frac{E_{520 \mu} - \frac{1}{2} (E_{460 \mu} + E_{580 \mu})}{\text{Corrected Value of } 17-KS \text{ in Material}} \times \frac{24 \text{ Hours' Total Urine}}{10} \times \frac{0.05 \text{ mg of DEAS}}{\text{Day}} \]

B. Thin Layer Chromatography of Urinary Total 17-KS

1. Hydrolysis extraction: One fiftyth volume of 24 hours’ total urine was used as the material. Reagents were used in the similar proportion as in the determination of urinary total 17-KS.

2. Preparation of chromatoplate: Using Kiesel gel G of Stahl, thin layer of 250 μ was produced, which was activated by leaving in hot air of 100°C for 30 minutes.

3. Application of 17-KS material on the chromatoplate: The material was dissolved in 0.05 cc of methanol on the heat, approximately 0.01 cc of which was dropped on the plate on the due spot using pipette.

4. Solvent for development: Chloroform of 97.8 cc and methanol of 2.2 cc. Breadth of development was adjusted to be 100 mm within 30 minutes.

5. Color was developed by spray of conc. H₂SO₄.

C. Determination of Urinary Total 17-OHCS²¹)²²)²³)²⁴) (Modified Method of Glenn & Nelson²³)

1. Ten cc of 24 hours’ total urine was used as the material.

2. Reagents

i) β-glucuronidase in concentration of 3300 Fishmann units in 1 cc.

ii) Sodium acetate solution of 0.2 mol

iii) Chloroform of special class

iv) 0.1 N NaOH solution

v) Na₂SO₄

vi) Ethyl alcohol of special class

vii) Conc. sulphuric acid as sulphuric acid reagent: Conc. sulphuric acid of 500 cc was added to 500 cc of distilled water under cooling.

viii) Phenylhydrazine sulphuric acid reagent: Phenylhydrazine hydrochloride of
65 mg was dissolved in 100 cc of sulphuric acid reagent, which was newly prepared every-
time at use.

ix) Standard solution: Hydrocortisone ethyl alcohol solution in concentration of 100 \( \gamma/\text{cc} \) was used, being appropriately diluted at use.

3. Determination of urinary total 17-OHCS

i) Hydrolysis: Ten cc of urine was put in a centrifugation tube and 1 cc of 0.2 mol sodium acetate solution and 1 cc of 0.2 mol sodium acetate glacialae were added and mixed. Ph was adjusted to be 4.5 to 4.8 using ph test paper (ph 3.8 to 5.4, Merk Co.), by dripping 50 per cent sodium acetate glacialae solution. Then, 0.3 cc of \( \beta \)-glucuronidase was added further and let stand in an incubator for 48 hours at a temperature of 47°C.

ii) Extraction: Chloroform of 7.5 cc was added to the above mentioned mixture and vigorously shaken for extraction. Since the separation was frequently difficult when urine of dogs was used as the material, the material was centrifuged for separation. This procedure of extraction was repeated twice and final outcome was 15 cc of chloroform containing the extract, which was rinsed with 2 cc of 0.1 N NaOH solution. The rinsed material was centrifuged for separation, and the material was again rinsed when still stained. It was furthermore rinsed twice with 1.5 cc of distilled water, and centrifuged for separation. The material was desiccated with 0.5 g of Na\(_2\)SO\(_4\), and dried in a temperature below 50°C under negative pressure.

iii) Colorimetry: Special class ethanol of 22 cc was added to the above mentioned dried material to dissolve, and it was divide into two test tubes, the amount being approximately 10 cc, respectively (there was decrease of 2 cc partly due to vaporization). In one test tube, 4 cc of phenylhydrazine sulfate reagent was added and this was regarded as sample A. In another test tube, 4 cc of sulphuric acid reagent was added and the latter was regarded as sample B. On the other hand, 10 cc of special class ethanol used as the solvent was respectively put in two test tubes, one being added with 4 cc of phenylhydrazin sulfate reagent as blank A, and another being added with 4 cc of sulphuric acid reagent as blank B. After these four test tubes were warmed in water bath of 60°C for 30 minutes, they were rapidly cooled down in streaming water, and the materials were put in the cuvettes of Beckmann spectrophotometer for determination of colorimetric absorption.

iv) Standard curve: Standard solution of hydrocortisone of 10 \( \gamma \), 20 \( \gamma \) and 30 \( \gamma \) was respectively taken and vaporized to dry up, which was similarly treated as the above described materials, and subjected to determination of colorimetric absorption. Colorimetric absorptions of each standard solution showed lineal correlation between the concentrations of the standard materials as shown in the figure, coefficient of absorption being

\[
\frac{A}{10} = \frac{B}{20} = \frac{C}{30}
\]
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v) Calculation:

\[
\begin{align*}
& a = \text{Sample A} - \text{Sample B} \\
& b = \text{Blank A} - \text{Blank B}
\end{align*}
\]

Difference between a and b was sought using filters of 410 mμ, 370 mμ and 450 mμ, and the values were respectively represented as p, q and r and

\[
2p - (q + r) \quad \text{was calculated.}
\]

Assuming the absorption coefficient of each material to be S₁, S₂ and S₃, urinary total 17-OHCS in X cc of 24 hours’ total urine is obtained from the following calculation.

\[
\frac{X_1}{5} \times S_1 \quad (\text{or} \quad S_2, \quad S_3, \ldots) \quad \text{Absorption coefficient being} \quad \frac{A}{10},
\]

\[
\frac{X_1S_1}{5} \times 10 = \frac{10X_1S_1}{5A} \gamma = 0.024 \times X_1S_1 \, \text{mg/day}
\]

D. Thin Layer Chromatography of Urinary Total 17-OHCS

1. Reagents: One fiftyth volume of 24 hours’ total urine of totally depancreatized dog was used.

2. Extraction: Reagents for extraction were used in the similar proportion as above described extraction of total 17-OHCS.

3. Preparation of chromatoplate: Kiesel gel G of Stahl was used and thin layer of 250 μ was produced.

4. Activation was carried out in hot air of 100°C for 30 minutes.

5. Solvent for development: The solvent was consisted of chloroform and ethanol in proportion of 93 : 7.

6. Time of development was 30 minutes.

7. Development of color was done by spray of a mixture solution of 4 cc of 0.2 per cent blue tetrazolium and 2 cc of 10 per cent NaOH.

E. Determination of Free 17-OHCS in Plasma (TAKEDA's method).

1. Reagents
   i) Dichloromethane of special class
   ii) 0.1 N NaOH
   iii) Ethanol H₂SO₄ solution: Twenty-five cc of ethanol and 75 cc of sulphuric acid was mixed.
   iv) Standard solution of hydrocortisol: Concentration of the solution was 1 γ per 0.1 cc.

2. Determination
   i) Five cc of heparinized frozen serum was added to 20 cc of dichloromethane and vigorously shaken for extraction using separation funnel.
   ii) Using separation funnel, the material was rinsed with 3 cc of 0.1 N NaOH solution.
   iii) Hydrocortisol standard solution of 0.2 cc was put in the cuvette.
   iv) For the blank, 20 cc of dichloromethane was taken into separation funnel.
   v) Ethanol sulphuric acid solution of 10 cc was added to develop fluorescence, and 17-OHCS was extracted into ethanol sulphuric acid solution.
   vi) Using standard solution of ethanol sulphuric acid solution, 0 and 100 points
of electric colorimeter were adjusted.

3. Calculation:
   Electric colorimeter was adjusted to indicate 100 per cent with 2γ of material and
   1 per cent with 0.02γ, and if blank shows 9 per cent and Material A shows 38 per
   cent:
   
   Free 17-OHCS in 5 cc of Plasma = (A-Blank) 0.02γ
   
   Free 17-OHCS in 100 cc of Plasma = (A-Blank) 0.02 × $\frac{100}{5}$ γ = (38-9) 0.4γ
   
   =11.6γ

   Attention was paid to finish the determination within 5 minutes after the ethanol
   sulphuric acid solution was added.

F. Eosinophil Count in Peripheral Blood
   Blood was aspirated into a mélangeur for white blood cell until the mark of 0.5, and
   eosinophils were stained by Hinkelmann's solution. Fuchs-Rosenthal chamber was used for
   the counting.

   Number of Eosinophils=6.25×Number of Eosinophils in Chamber

G. Thorn Test
   1. After blood sample was taken, 10 units of ACTH was intramuscularly injected.
   2. Blood was taken again 5 hours after injection, and eosinophils were counted
      in peripheral blood.
   3. Water and food were given to the animals and extremities were not fixed.

   Rate of Decrease in Eosinophils
   = $\frac{\text{Eosinophil Count before Injection} - \text{Eosinophil Count 5 Hours Later}}{\text{Eosinophil Count before Injection}} \times 100$ (%)

H. Histological Study of Adrenal Cortex
   1. Hematoxylin-eosin double staining: The fixed sections were embedded in
      paraffin.
   2. Sudan III staining: The fixed sections were embedded in carbowax.

I. Ratio of Adrenal Weight to Body Weight
   Immediately after death, animals were autopsied, and the adrenal gland was fixed in
   10 per cent neutral formalin. The adrenal gland was weighed 24 hours later.

III. RESULTS

A. Fluctuation of Urinary Total 17-KS in Totally Depancreatized Dogs
   As summarized in Tab. 1 and Fig. 1, preoperative average value of total 17-KS in
   urine in the present experiment was 0.53 mg/day (0.35 mg/day to 0.76 mg/day). After
   total pancreatectomy, urinary total 17-KS increased in all groups, without administration
   of drug, with estrogen administration, with insulin administration and simultaneous admin-
   istration of estrogen and insulin, reaching its maximum level around 2 or 3 weeks after
   surgery, and it showed a tendency of decrease thereafter. In the group without admini-
   stration of drug, urinary total 17-KS increased as much as to be 1.72 mg/day 2 weeks
   after total pancreatectomy, which was the maximum value in all the experimental groups.
   In the group of estrogen administration, the peak was observed to be 1.43 mg/day 2 weeks
   after surgery. The maximum level was observed 3 weeks after surgery to be 1.32 mg/
INFLUENCE OF ESTROGEN ON DEPANCREATEIZED DOGS

Table 1 Fluctuation of Urinary Total 17-KS mg/day

<table>
<thead>
<tr>
<th>Dog</th>
<th>Before</th>
<th>1 W</th>
<th>2 W</th>
<th>3 W</th>
<th>4 W</th>
<th>5 W</th>
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<td>0.50</td>
<td>0.56</td>
<td>0.63</td>
<td>1.08</td>
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<td>0.60</td>
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<tr>
<td>C2</td>
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<td>0.47</td>
<td>0.64</td>
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<td>0.49</td>
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<tr>
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<td>0.60</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>C22</td>
<td>0.38*</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C21</td>
<td>0.44</td>
<td>0.50</td>
<td>0.60</td>
<td>1.08</td>
<td>0.99</td>
<td>0.60</td>
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<tr>
<td>Mean</td>
<td>0.49</td>
<td>0.50</td>
<td>0.60</td>
<td>1.08</td>
<td>0.99</td>
<td>0.60</td>
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<tr>
<td>I2</td>
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</tr>
<tr>
<td>Mean</td>
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<td>1.28</td>
<td>1.32</td>
<td>1.19</td>
<td>0.81</td>
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<td>E10</td>
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<td>E17</td>
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<td>1.08</td>
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<tr>
<td>E23</td>
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<td></td>
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<td>1.78*</td>
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<td>1.43</td>
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<td></td>
<td>1.00</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>0.50</td>
<td>1.00</td>
<td>1.82</td>
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<td></td>
</tr>
<tr>
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<td>0.76</td>
<td>1.54</td>
<td>2.35*</td>
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<tr>
<td>Mean</td>
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<td>1.27</td>
<td>1.72</td>
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</table>

Total Mean | 0.53 |

*One and the same material urine with Thin Layer Chromatography of Fig. 3 and Fig. 18.

Fig. 1 Fluctuation of Urinary Total 17-KS mg/day

day in the group of insulin administration. In the group of simultaneous administration of estrogen and insulin, the peak was observed to be 1.08 mg/day 3 weeks after surgery. Thus, in the group of estrogen and insulin administration, appearance of peak of urinary
total 17-KS level was the most retarded and degree of increase also the slightest compared with other 3 groups, and it is assumed that in this group fluctuation of urinary total 17-KS level caused by total pancreatectomy was the slightest compared with any other groups.

B. Fluctuation of Urinary Total 17-KS in Totally Depancreatized Patient

Fluctuation of urinary total 17-KS was investigated in a 55-year-old female, whose pancreas was totally removed due to cancer in the head of the pancreas. As shown in Fig. 2, urinary total 17-KS level was 5.22 mg/day preoperatively, 4.20 mg/day 1 week after surgery and 9.35 mg/day 2 weeks after surgery. Twenty units of insulin was daily administered to this patient until 2 weeks after surgery. However, as 10,000 units of estrogen was additionally administered everyday later than 2nd postoperative week, urinary total 17-KS level fluctuated to be 0.66 mg/day 3 weeks after surgery, 1.20 mg/day 4 week after surgery, 2.09 mg/day 5 weeks after surgery, 1.50 mg/day 6 weeks after surgery and 1.00 mg/day 8 weeks after surgery, ranging below average value in healthy human (3 mg/day to 5 mg/day) with favorable general condition.

C. Thin Layer Chromatography of Urinary Total 17-KS in Totally Depancreatized Dogs

One fiftieth volume of 24 hours’ total urine was used as material. As shown in Tab. 2 and Fig. 3, among fractions of thin layer chromatography of preoperative urinary total 17-KS, the fraction which showed relatively large amount was androsterone (Rf 60, with violet blue tincture), ranging 6.9 γ. On the chromatography of urinary total 17-KS 2 weeks after total pancreatectomy, methyltestosterone (Rf 53, with reddish yellow tincture), which could not be observed preoperatively, showed marked increase in group without administration of drug, group of estrogen administration, group of insulin administration and group of estrogen and insulin administration, ranging 2.3 γ in C 22 (Dog No. 22 in
INFLUENCE OF ESTROGEN ON DEPANCREATEIZED DOGS

Table 2 Thin Layer Chromatography of Urinary Total 17-KS

<table>
<thead>
<tr>
<th>Material</th>
<th>Urine cc</th>
<th>24 hours' total Urine</th>
<th>Total 17-KS</th>
<th>17-KS</th>
<th>17-KS</th>
</tr>
</thead>
<tbody>
<tr>
<td>C22</td>
<td>950</td>
<td>950</td>
<td>0.38</td>
<td>0.54</td>
<td>600</td>
</tr>
<tr>
<td>C22</td>
<td>850</td>
<td>1950</td>
<td>1.22</td>
<td>1.78</td>
<td>2.35</td>
</tr>
<tr>
<td>I21</td>
<td>1850</td>
<td>21</td>
<td>39</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>E23</td>
<td>600</td>
<td>600</td>
<td>21</td>
<td>12</td>
<td>cc</td>
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<tr>
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<td>11050</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24.4</td>
<td>35.6</td>
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<tr>
<td>Androsterone</td>
<td>6.9</td>
<td>6.9</td>
<td>4.1</td>
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<tr>
<td>Methyltestosterone</td>
<td>7.3</td>
<td>7.3</td>
<td>1.8</td>
<td>2.7</td>
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</tr>
</tbody>
</table>

Material Urine = 24 hours' total Urine x 1/50
Kieselgel G 250 μ
Time of development 30 min.

Fig. 3 Thin Layer Chromatography of Urinary Total 17-KS in Totally Depancreatized Dogs

D. Fluctuation of Urinary Total 17-OHCS in Totally Depancreatized Dogs

Urinary total 17-OHCS was determined following modified method of Glenn and Nelson. As shown in Tab. 3 and Fig. 4, preoperative average value in normal dogs
Table 3  Fluctuation of Urinary Total 17-OHCS mg/day

<table>
<thead>
<tr>
<th>Group of Estrogen and Insulin Administration</th>
<th>Dog</th>
<th>Befor</th>
<th>1 W</th>
<th>2 W</th>
<th>3 W</th>
<th>4 W</th>
<th>5 W</th>
</tr>
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<tbody>
<tr>
<td>C21</td>
<td>$\beta$</td>
<td>0.32</td>
<td>0.50</td>
<td>0.23</td>
<td>0.71</td>
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<tr>
<td>C22</td>
<td>$\beta$</td>
<td>0.26</td>
<td>0.37</td>
<td>0.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C23</td>
<td>$\beta$</td>
<td>0.29</td>
<td>0.37</td>
<td>0.71</td>
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<tr>
<td>C8</td>
<td>$\beta$</td>
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Total Mean                                 |     | 0.30 | 0.51 | 1.44 |

* One and the same material urine with Thin Layer Chromatography of Fig. 5 and Fig. 19
+ One and the same material urine with Thin Layer Chromatography of Fig. 6

Fig. 4  Fluctuation of Urinary Total 17-OHCS mg/day
was 0.3 mg/day (0.26 mg/day to 0.38 mg/day). The value increased in all groups after total pancreatectomy, and seemed to reach its peak 3 to 4 weeks after surgery and to decrease thereafter. In the group without administration of drug, the value reached the highest level of 1.34 mg/day 2 weeks after surgery. The peak was observed 3 weeks after surgery in the group of estrogen administration to be 0.99 mg/day, 3 weeks after surgery in the group of insulin administration to be 0.95 mg/day and 4 weeks after surgery in the group of estrogen and insulin administration to be 0.71 mg/day. Thus, urinary total 17-OHCS level was the highest in the group without administration of drug, its peak appearing early, and appearance of the peak was retarded with lower level in the order of the group with estrogen administration and the group with insulin administration. In addition, this tendency was the most marked in the group of estrogen and insulin administration.

E. Thin Layer Chromatography of Urinary Total 17-OHCS in Totally Depancreatized Dogs

It was recognized that main fractions in thin layer chromatography of 17-OHCS of normal dog were contained in F of Rf 30 to 35 (Hydrocortisone), and another fraction was also observed in Rf 90 to 100. Fractions of 17-OHCS 2 weeks after total pancreatectomy showed markedly diversified findings. Namely, there appeared fractions of THF (tetrahydrocortisol) and THE (tetrahydrocortisone), and there appeared also fractions of S (11-desoxycortisol) and E (Cortisone) in E 21 (Dog No. 21 in group of estrogen administration), F 23 (Dog No. 23 in group without administration of drug) and E 23 (Dog No. 23 in group of estrogen administration). Fractions of Rf 90 to 100, which

![Fig. 5 Thin Layer Chromatograph of Urinary Total 17-OHCS in Totally Depancreatized Dogs]
could be observed in normal dogs, could also be observed in the postoperative thin layer chromatography. To summarize these findings, it is assumed that sorts and quantity of the appearing fractions were the most abundant in the group without administration of drug, which decreasing in the order of group with estrogen administration and group with insulin administration, and these were the least in the group of simultaneous administration of estrogen and insulin.

F. Fluctuation of Free 17-OHCS in Plasma in Totally Depancreatized Dogs

Plasma 17-OHCS was determined following TAKEDA's ethanol sulphuric acid fluorescence method. Preoperative average value was 10.6 γ/100 cc (8.4 γ/100 cc to 11.6 γ/100 cc), as shown in Tab. 4 and Fig. 7. The value increased in all groups after total pancreatectomy, reaching its peak by approximately 4th postoperative week and decreasing gradually thereafter.

Free 17-OHCS in plasma was 19.1 γ/100 cc in the group without administration of drug 2 weeks after surgery, showing the highest level among all 4 groups. It was 12.8 γ/100 cc in the group of estrogen administration 2 weeks after surgery, 11.6 γ/100 cc in the group of insulin administration 2 weeks after surgery and 11.2 γ/100 cc in the group of estrogen and insulin administration. Such tendency was similarly observed in 3rd and 4th postoperative weeks, i.e. the value being 19.0 γ/100 cc in the group of estrogen administration, 14.3 γ/100 cc in the group of insulin administration and 13.7 γ/100 cc in the group of estrogen and insulin administration, respectively 4 weeks after surgery. In the group without administration of drug, early and marked increase in plasma 17-OHCS was observed 2 weeks after surgery, and the increase was slighter in the order of group of estrogen administration, group of insulin administration and group of simultaneous administ-
INFLUENCE OF ESTROGEN ON DEPANCREATIZED DOGS

Table 4 Fluctuation of Plasma 17-OHCS γ/100cc

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Total Mean | 10.6 γ/100cc

Fig. 7 Fluctuation of Free 17-OHCS in Plasma γ/100cc

G. Fluctuation of Eosinophil Count in Peripheral Blood in Totally Depancreatized Dogs

Average eosinophil count of peripheral blood in normal dogs was observed to be 813/
mm³ (268/mm³ to 2806/mm³) as shown in Tab. 5 and Fig. 8, and it was noticeable that individual deviation was so large. After total pancreatectomy, eosinophil count decreased in all the groups, without administration of drug, with estrogen administration, with insulin administration and with simultaneous administration of estrogen and insulin. In the group without administration of drug, early and marked decrease in eosinophil count to be 31/mm³ 2 weeks after surgery. The decrease was slightly less to be 181/mm³ in the group of estrogen 3 weeks after surgery. It was more slight in the group of insulin administration to be 448/mm³ 3 weeks after surgery, and it was the smallest in the group of estrogen and insulin administration to be 695/mm³ 3 weeks after surgery. In the group of estrogen and insulin administration, eosinophil count showed the slightest decrease to be 575/mm³ 7 weeks after total pancreatectomy. Hence, it is assumed that the fluctuation of eosinophil count in peripheral blood shows close correlation to that of urinary 17-KS, urinary 17-OHCS and plasma 17-OHCS. In brief, both 17-KS and 17-OHCS

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Total Mean 813/mm³ stained by Hinkelmann's solution.
increased the most conspicuously in the group without administration of drug compared with any other groups, being accompanied by marked decrease in eosinophil count, and increase in 17-KS and 17-OHCS and decrease in eosinophil count being less in the order of group of estrogen administration, group of insulin administration and group of simultaneous administration of estrogen and insulin.

H. Fluctuation of Response to Thorn Test in Totally Depancreatized Dogs

As shown in Tab. 6 and Fig. 9, average value of preoperative Thorn test in totally depancreatized dogs was 60 per cent (52 to 66 per cent). Average value of Thorn test
2 weeks after total pancreatectomy was 60 per cent in the group of insulin administration and 55 per cent in the group of estrogen and insulin administration remaining within normal range or being accompanied by only a slight decrease, whereas it was 17 per cent in the group of estrogen administration and in the group without administration of drug it was in as low a level as 23 per cent 1 week after total pancreatectomy, both intimately reflecting profound impairment of adrenocortical reserve capacity.

I. Ratio of Adrenal Weight to Body Weight
As represented in Tab. 7 and Fig. 10, proportion of adrenal weight to body weight in normal animals was 0.19 g/kg body weight, on the average. In totally depancreatized dogs, it was 0.18 g/kg body weight in the group of estrogen and insulin administration and 0.17 g/kg body weight in the group of insulin administration, showing lower level

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</table>
Fig. 10 Fluctuation of Adrenal Weight to Body Weight after total pancreatectomy

than in normal animals. On the contrary, it was in such a high level as 0.23 g/kg body weight in the group of estrogen administration and 0.24 g/kg body weight in the group without administration of drug.

J. Histological Findings of Adrenal Cortex in Totally Depancreatized Dogs

Thickness of each zone of the adrenal cortex in normal dogs was 1.0 in zona glomerulosa, 6.0 in zona fasciculata and 0.5 in zona reticularis, as shown in Tab. 8. In totally depancreatized dogs, changes could be observed in thickness of each zone in the four experimental groups. Thickness of zona fasciculata in I 2 (Dog No. 2 in group of insulin administration) was 5.5 and in I 3 (Dog No. 3 in group of insulin administration) it was 5.0, showing an atrophic change, whereas in the group of estrogen administration,
Table 8 Comparison in Adrenocortical thickness after total pancreatectomy

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</tbody>
</table>

thickness of zona glomerulosa was 2.0 in E 1 (Dog No. 1 in group of estrogen administration) and 1.5 in E 10 (Dog No. 10 in group of estrogen administration), and thickness of zona fasciculata was 10.0 in E 1 (Dog No. 1 in group of estrogen administration) and 7.0 in E 10 (Dog No. 10 in group of estrogen administration), and thickness of zona reticularis was 4.0 in E 1 and 1.2 in E 10 (Dog No. 1 and 10, respectively in group of estrogen administration), showing a hypertrophic change in all 3 zones of the adrenal cortex. In group of estrogen and insulin administration, the thickness was 2.0 and 1.3 in zona glomerulosa with hypertrophic change, 4.0 and 4.0 in zona fasciculata with atrophic change and 0.8 and 1.0 in zona reticularis with hypertrophic change. It is noteworthy that the finding in the group of estrogen and insulin administration is nothing but a sum of findings in the groups of estrogen administration and insulin administration. In the group without administration of drug, the thickness of zona glomerulosa showed narrowing ranging 0.8 or 0.4, while the thickness of zona reticularis showed hypertrophic change ranging 2.0 and 2.0. Narrowing of zona glomerulosa which was found in the group without administration of drug was the only finding among four groups of experiment, which was assumed to suggest peculiarity of the metabolism.

Concerning the fat granule in the adrenal cortex in totally depancreatized dogs, decrease in sudanophile granules in Sudan III staining could not be observed, but reddish granules could be observed, as shown in Fig. 11, in C 1 (Dog No. 1 in the group of estrogen and insulin administration) which died of ileus 35 days after surgery. As has been pointed out, attitude of fat granule in the group of insulin administration after total pancreatectomy shows some specific character. In I 1 (Dog No. 1 in the group of insulin administration), which died of insulin shock 9 days after surgery, increase in sudanophile granules was represented by strongly stained reddish granules, as shown in Fig. 12. Thus, in the group of insulin administration, temporary increase in sudanophile granules was observed 1 to 2 weeks after total pancreatectomy, which was assumed to be specific. In I 3 (Dog No. 3 in the group of insulin administration), which died of insulin shock 25 days after surgery, decrease in sudanophile granules was observed and they are represented by brown ones as shown in Fig. 13. Thus, more than 2 weeks after surgery there ap-
peared decrease in sudanophile granule in the group of insulin administration. Temporary increase in fat granule as observed postoperatively in the group of insulin administration could not be observed in the group of estrogen administration. In Sudan III staining, the adrenocortex was stained brown showing decrease in sudanophile granules in E 10 (Dog No. 10 in the group of estrogen administration) which died 12 weeks after surgery due to emaciation, as shown in Fig. 14. It was observed that sudanophile granules in the group of estrogen administration decrease on in one way.

It is noteworthy that adrenocortex was stained brown in Sudan III staining showing marked decrease in sudanophile granules in F 1 (Dog No. 1 in the group without administration of drug), as shown in Fig. 15, despite it died of cachexia in early period of 14 days after surgery. At the same time, marked change in structural cells was also characteristic.

Concerning cellular arrangement in adrenocortex of totally depancreatized dogs, transition of outer area in zona fasciculata into the structure of zona glomerulosa was observed as shown in Fig. 11 in C 1 (Dog No. 1 in the group of estrogen and insulin administration), and the likewise transition could be observed in the same area as shown in Fig. 17 in E 12 (Dog No. 12 in the group of estrogen administration). On the other hand, transition of inner area in zona glomerulosa into the structure of zona fasciculata was observed in F 1 (Dog No. 1 in the group without administration of drug) as shown in Fig. 15.

Finding of capsule adenoma was observed as shown in Fig. 16 in E 3 (Dog No. 3 with estrogen administration in a dose of 10,000 units per kilogram body weight) when it died 26 days after total pancreatectomy, as well as in E 10 (Dog No. 10 with estrogen administration of 1,000 units per kilogram body weight) when it died 2 weeks after surgery. It is reported by AIKAWA that capsule adenoma develops also when treated with insulin alone after total pancreatectomy. In the present experiment, capsule adenoma could be observed in I 1 (Dog No. 1 with insulin administration in a dose of 2 units per kilogram body weight) which died 9 days after total pancreatectomy, and this was also observed in F 1 (Dog No. 1 in the group without administration of drug) which died 2 weeks after surgery. Development of capsule adenoma could not be observed in the group of estrogen and insulin administration.

IV. DISCUSSION

Urinary total 17-KS and urinary total 17-OHCS were determined in totally depancreatized dogs, and urinary total 17-KS and urinary total 17-OHCS obtained 2 weeks after surgery from totally depancreatized dogs were developed on thin layer chromatography. Quantitative fluctuation of these two on the thin layer chromatography behaved in parallel with each other. Fluctuation of urinary 17-KS and urinary 17-OHCS showed similar tendency to each other in the group without administration of drug, in the group of estrogen administration, in the group of insulin administration and in the group of simultaneous administration of estrogen and insulin, as shown in Fig. 1 and Fig. 4, as well as fluctuation of 17-OHCS in plasma, as shown in Fig. 7.

BUCHUS postulated that 17-OHCS turns into 17-KS in the liver, and in 1951, BONGIOVANNI and WILLIAMS reported that urinary 17-KS decreases in cases of hepatic diseases. On the other hand, TANIGUCHI reported an occurrence of fatty liver after total
pancreatectomy. In the present experiment, urinary total 17-KS level was low being 1.00 mg/day 2 weeks after total pancreatectomy in F1 (Dog No. 1 in the group without administration of drug) which developed fatty liver, and marked increase in urinary total 17-KS was observed to be 2.35 mg/day 2 weeks after total pancreatectomy in F23 (Dog No. 23 in the group without administration of drug) which showed no evidence of fatty liver development. In this respect, the results of the present experiment coincide with the assertion of Bongiovanni.

Nishikawa reported average value of urinary total 17-KS in normal dogs to be 0.46 mg/day and it was 0.53 mg/day in the present experiment. Both urinary 17-KS and urinary 17-OHCS levels are lower in dogs than in men, being approximately one tenth of the latter, and in the extraction procedure separation of urine from the reagents is more difficult. Consequently, as mentioned in the above, the methods were partly modified, since experimental data could not be obtained when the original methods were followed.

Wintersteiner and Pfiffner observed that cortisol and corticosterone exhibit fluorescence by the aid of sulphuric acid, which was utilized by Sweat in 1953 for determination of cortisol in blood, and this method of determination has been variously modified and improved. In the present experiment, 17-OHCS in blood was determined following the method of Takeda. Preoperative average value of 17-OHCS in totally depancreatized dogs was 10.6 γ/100 cc in the present experiment and 7.7 γ/100 cc in the report of Akawa, though the method of determination is different between these two.

As described in the above, considerably distinct correlation could be observed between eosinophil count in peripheral blood and the data of urinary total 17-KS, urinary total 17-OHCS and 17-OHCS in plasma. Hence, it is assumed that eosinophil count in peripheral blood might well reflect general condition of totally depancreatized dogs. As shown in Fig. 8, marked decrease in eosinophil count could be observed in early postoperative period in the group without administration of drug, the count being 31/mm³. The degree of decrease in eosinophil count was the less as general condition became the more favorable in the order of the group of estrogen administration, the group of insulin administration and the group of estrogen and insulin. The eosinophil count in the group of estrogen and insulin administration was determined to be 575/mm³ 7 weeks after total pancreatectomy. From a simple investigation on eosinophil count, it could be presumed that administration of estrogen or insulin of suitable dose can lessen the stress of total pancreatectomy as well as simple administration of each drug and simultaneous administration of these two.

Concerning the cause of decrease in eosinophil count, various assertions have been made. In the present experiment, it was interpreted that degree of decrease in eosinophil count was corresponding to the amount of 17-OHCS released into blood stream, depending on the degree of stress due to total pancreatectomy in each group of the experiment. It was, furthermore, assumed that such increase in the release of 17-OHCS into blood stream is presumably due to pathologic hyperfunction of the adrenal cortex.

Preoperative average value of Thorn test was reported by Akawa to be 87 per cent, and it was 60 per cent in the present experiment. This difference in the average value might probably due partly to the method of administration of ACTH of 10 units, intravenously in the former and intramuscularly in the latter, and partly to the fact that
Fig. 11  C 1
Sudan III ×100
Died of intestinal obstruction 35 days after total pancreatectomy

Fig. 12  I 1
Sudan III ×100
Died of insulin shock 9 days after total pancreatectomy

Fig. 13  I 3
Sudan III ×100
Died of insulin shock 25 days after total pancreatectomy

Fig. 14  E 10
Sudan III ×100
Died of cachexia 12 weeks after total pancreatectomy

Fig. 15  F 1
Sudan III ×100
Died of cachexia 14 days after total pancreatectomy

Fig. 16  E 3
H-E ×100
Capsule adenoma:
Died of cachexia 26 days after total pancreatectomy
Fig. 17 E 12 H-E ×100
Died of cachexia 5 weeks after total pancreatotomy

Fig. 18 Thin layer chromatography of urinary total 17-KS in totally depancreatized dog

Fig. 19 Thin layer chromatography of urinary total 17-OHCS in totally depancreatized dog
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in the former experiment, the animals were fixed by their extremities whereas in the latter they were not fixed and animals could take food AD LIBITUM.

As shown in Fig. 9, Thorn test in the group without administration of drug revealed marked fall of adrenocortical reserve, and this finding was assumed to represent the fact that the animals endeavored for themselves to resist endocrine insufficiency brought about by total pancreatectomy and endocrine balance was just to be ruined being exhausted out. This interpretation well coincides with the histological finding of the adrenocortex, such as vacuole formation in the cells and transformation of each zones.

Concerning the relationship between adrenocortical reserve and sudanophile granules in the adrenocortex, temporary increase in sudanophile granules was observed 2 weeks after total pancreatectomy in the group of insulin administration, as reported by Nomura and Aikawa, and the result of Thorn test was, as shown in Fig. 9, 60 per cent 2 weeks after surgery in the group of insulin administration, which was higher than 55 per cent in the group of estrogen and insulin administration. Moreover, as shown in Tab. 2, on the thin layer chromatography of urinary total 17-KS in the group of insulin administration performed 2 weeks after surgery, amount of androsterone was 7.3 γ and that of methyltestosterone was 1.8 γ, while the former being 4.1 γ and the later being 2.3 γ in the group of estrogen and insulin administration. It is a noteworthy finding that in the group of insulin administration, androsterone, which is contained in urinary total 17-KS of normal dogs, so to say a normal fraction, was contained more and methyltestosterone, which is accepted to be a product of pathologic metabolism, was contained less compared with the group of estrogen and insulin administration. On the other hand, as shown in Fig. 12, histologic examination of adrenocortex carried out in I 1 (Dog No. 1 in the group of insulin administration) 2 weeks after surgery revealed increase in sudanophile granules, which were more strongly stained reddish than in C 1 (Dog No. 1 in the group of estrogen and insulin administration), as shown in Fig. 11. From this finding, a close relationship between androsterone fraction level and sudanophile granule can be inferred. In other words, adrenocortical reserve is preserved within sudanophile granules, and it is presumed that secretion of androsterone is largely influenced by sudanophile granules in the adrenal gland. O'Donnell reported in 1951 that administration of ACTH in men resulted in hypertrophy of zona reticularis, narrowing of zona glomerulosa and obscuration of transitional zone in the adrenocortex. Similar findings could be observed, as shown in Tab. 8, in the group without administration of drug. Outstanding change was observed, as demonstrated by Hatta, in the histologic examination of the hypophysis in the group without administration of drug, suggesting prosperous release of ACTH. This is assumed to be a marked pathologic hyperfunction of the hypophysis-adrenal system.

In the present experiment, Kiesel gel G was used for thin layer chromatography in a thickness of 250 μ. When mixed solution of 97.8 cc of chloroform and 2.2 cc of methanol was used for development solvent of urinary total 17-KS, fractions of Rf 90 to 100 were favorably developed as shown in Fig. 3, and when mixed solution of 93 cc of chloroform and 7 cc of ethanol was used for development solvent of urinary total 17-OHCS, fractions of Rf 90 to 100 were favorably developed as shown in Fig. 5.
V. SUMMARY

Total pancreatectomy was performed experimentally in dogs and adrenocortical function was investigated 1) in the group without administration of drug, 2) in the group of estrogen administration in the dosage of 1,000 units per kg body weight, 3) in the group of insulin administration in the dosage of 2 units per kg body weight and 4) in the group of simultaneous administration of estrogen of 1,000 units and insulin of 2 units, respectively per kg body weight. The obtained results are summarized as follows.

1. Urinary total 17-KS, urinary total 17-OHCS and free 17-OHCS in plasma increased in all groups of the experiment reaching their peak 2 to 4 weeks after total pancreatectomy, which were followed by gradual decrease thereafter. Increase in 17-KS and 17-OHCS was most remarkable in the group without administration of drug among all 4 groups, and in this group the maximum levels appeared earliest than any other groups. The degree of the increase was less in the order of the group of estrogen administration, the group of insulin administration and the group of estrogen and insulin administration, and the appearance of the maximum level was also retarded in this turn. From these findings, it can be said that the influence of total pancreatectomy was the minimum in the group of estrogen and insulin administration among these 4 groups of the experiment.

2. On the thin layer chromatography of urinary total 17-KS of normal dog, androsterone fraction occupied the most part, while on the thin layer chromatography of urinary total 17-KS from totally depancreatized dogs 2 weeks after surgery, there appeared methyltestosterone fraction which could not be observed preoperatively, and violet blue fraction was also observed in Rf 22 in the group of estrogen administration and the group without administration of drug.

It was observed that the thin layer chromatography of urinary total 17-OHCS of normal dogs was principally consisted of hydrocortisone fraction. There appeared tetrahydrocortisol fraction and tetrahydrocortisone fraction on the thin layer chromatography of urinary total 17-OHCS from totally depancreatized dogs 2 weeks after surgery, and in the group of estrogen administration and the group without administration of drug there appeared 11-desoxycortisol fraction and cortisone fraction. Thus, it was observed in the thin layer chromatographic study of urinary total 17-KS and urinary total 17-OHCS of totally depancreatized dogs that kinds of the fractions were the most various and their increase was the most remarkable in the group without administration of drug, which were less in the order of the group of estrogen administration and the group of insulin administration, and in the group of estrogen and insulin administration kinds of the fractions and their increase were the least.

3. Eosinophil count in peripheral blood decreased postoperatively in all 4 groups, and the most marked decrease appeared the earliest in the group without administration of drug, whereas the decrease was less in the order of the group of estrogen administration and the group of insulin administration, and the decrease was the slightest for long period in the group of estrogen and insulin administration, suggesting the minimum influence of total pancreatectomy on this group compared with any other groups.

4. Preoperative average value of Thorn test in totally depancreatized dogs was 60 per cent. It was 60 per cent 2 weeks after total pancreatectomy in the group of insulin
administration and 55 per cent in the group of estrogen and insulin administration, revealing slight decrease in adrenocortical reserve, while it was 17 per cent 2 weeks after total pancreatectomy in the group of estrogen administration and 23 per cent 1 week after surgery in the group without administration of drug, suggesting marked decline of adrenocortical reserve in these groups.

5. Ratio of adrenal weight to body weight in normal dogs was 0.19 g per kg, on the average. In totally depancreatized dogs, it was 0.18 g per kg body weight, on the average, in the group of estrogen and insulin administration and 0.17 g per kg body weight in the group of insulin administration, both showing small value. On the other hand, it was 0.23 g per kg body weight, on the average, in the group of estrogen administration and 0.24 g per kg body weight in the group without administration of drug, both showing an increase in the value.

It was presumed that the ratio of adrenal weight to body weight might be correlated with the thickness of each zone of the adrenocortex. In the group of insulin administration, narrowing was observed in zona fasciculata, showing small value of the ratio, and in the group of estrogen and insulin administration, hypertrophy of zona glomerulosa, narrowing of zona fasciculata and hypertrophy of zona reticularis were observed, with small value of the ratio. In the group of estrogen administration, hypertrophy was observed in three zones of zona glomerulosa, zona fasciculata and zona reticularis, with increase in the ratio, and hypertrophy was observed in zona reticularis in the group without administration of drug, similarly with increase in the ratio.

6. Histological study of adrenocortex was carried out in totally depancreatized dogs. By Sudan III staining, decrease in sudanophile granule could not be observed for long period in the group of estrogen and insulin administration, and the slice section was stained reddish. In the group of insulin administration, temporary increase in sudanophile granules was observed 1 to 2 weeks after total pancreatectomy, the slice being strongly stained reddish, but sudanophile granules decreased gradually more than 2 weeks after surgery, the slice being stained brown. In the group of estrogen administration, decrease in sudanophile granules was observed after surgery, the slice being stained brown. In the group without administration of drug, decrease in sudanophile granules was observed after surgery, the slice being stained brown, and devastation of the tissue was marked in the early postoperative period.

7. Close interrelation between adrenocortical reserve and sudanophile granules in the adrenocortex can be pointed out from the result that the findings of Thorn test and sudanophile granules were more favorable in the group of insulin administration than in the group of estrogen and insulin administration 2 weeks after total pancreatectomy.

8. Androsterone fraction, which exists in urinary total 17-KS of normal dogs, can be assumed to be a normal fraction. Close correlation was presumed between androsterone fraction amount and reddish stained sudanophile granules in the adrenocortex. On the other hand, methyltestosterone fraction, which appears after total pancreatectomy can be assumed to be a pathologic fraction, and it was presumed that there exists close correlation between methyltestosterone fraction amount and brown stained sudanophile granules in the adrenocortex. On the thin layer chromatography of urinary total 17-KS 2 weeks after total pancreatectomy, amount of androsterone fraction was 7.3γ in the group of
insulin administration, which was higher than the value of 4.1 \( \gamma \) in the group of estrogen and insulin administration, and in the finding of sudanophile granules also, the granules showed an increase in number and were more strongly stained reddish in the group of insulin administration than in the group of estrogen and insulin administration.

On the other hand, amount of methyltestosterone fraction, in the thin layer chromatography of urinary total 17-KS 2 weeks after total pancreatectomy, was 6.1\( \gamma \) in the group without administration of drug, showing markedly higher value compared with 2.7 \( \gamma \) in the group of estrogen administration, 1.8 \( \gamma \) in the group of insulin administration and 2.1 \( \gamma \) in the group of insulin and estrogen administration, and sudanophile granules were more strongly stained brown in the group without administration of drug than in other groups. It is assumed that sudanophile granules in adrenocortex are strongly stained reddish by Sudan III staining when androsterone fraction level is high; When the level of androsterone fraction is low, sudanophile granules are lightly stained red. On the other hand, when the level of methyltestosterone fraction is high, sudanophile granules are strongly stained brown, and they are lightly stained brown when the level of methyltestosterone fraction is low.

9. After total pancreatectomy, among urinary total 17-KS, androsterone fraction, which is assumed to be a normal fraction, showed a tendency of slightly decreased secretion, and secretion of methyltestosterone and other fractions as those in Rf 22 was markedly increased. This phenomenon was interpreted to represent depressed physiologic function and the state of pathologic hyperfunction.

From these findings, it is assumed that estrogen and insulin, even when administered respectively alone after total pancreatectomy, act to lessen the stress of the surgery, and when these two are simultaneously administered, unfavorable influence of total pancreatectomy can be well prevented, the animals being kept in a favorable condition for long period after surgery.

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VI. REFERENCES


和文抄録

腎全剝犬の副腎皮質に及ぼす Estrogen の影響

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腎全剝犬の病態生理は内分泌としての腎臓が欠乏するだけでなく、内分泌として体外のInsulinの全く存在しない点で、一部体外のInsulinの存在する腎部分切除と著しく異なっている。著者は腎全剝犬を作成し、従来行なわれて来たInsulin投与群に加えて、薬剤無投与群、Estrogen群、Estrogen Insulin併用投与群の4群とし、各種副腎皮質機能試験を行ない、比較検討を次に成績を得た。

1. 腎全剝犬の尿中17-KS、尿中17-OHCS、血中17-OHCSは術後各群とも増加し、2～4週をピークとし、以後は次第に下げる傾向を示すが、薬剤無投与群は増加が著明でピークの発現も早期であるが、Estrogen群、Insulin群、Estrogen Insulin併用群の順に増加も少なくなり、ピークの発現も遅延する傾向を認めた。

2. 正常犬の尿中17-KSの薄層クロマトグラフィーではAndrosterone分画が主をなすことを利用し、腎全剝犬の術後2週目の尿中17-KSの薄層クロマトグラフィーで術前で認め得なかった、Methyltestosterone分画の出現を認めた。尚 Estrogen群、薬剤無投与群ではRf 22に紫青の分画を認めた。

正常犬の尿中17-OHCSの薄層クロマトグラフィーではHydrocortisone分画が主をなすことを利用し、腎全剝犬の術後2週目の尿中17-OHCSの薄層クロマトグラフィーではTetrahydrocortisone分画、Tetrahydrocortisol分画の出現を認めた。また Estrogen群、薬剤無投与群では11-deoxy cortisol分画、Cortisone分画の出現を認めた。

即ち腎全剝犬の尿中17-KS、尿中17-OHCSの薄層クロマトグラフィーで薬剤無投与群では出現する分画の種類も多く、増量も多いかが、Estrogen群、Insulin群の順に分画の種類、量ともに少なくなり、Estrogen Insulin併用群では最も少なくなる傾向を認めた。

3. 腎全剝犬の末梢血の好酸球数は術後4週とも著減少するが、薬剤無投与群では早期に著減し2週目31/
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mm²となるに反し，Estrogen群，Insulin群の順に減少が少なくな、Estrogen Insulin併用群では長期に亘り減少が少なく7週目575/mm²を記録した。

4. Thorn氏試験では脾全剝去犬の前平均値は60%であった。術後2週目Insulin群60%，Estrogen Insulin併用群55%であり副腎皮質予備能の正常或は軽度の低下を認めたのに反し，Estrogen群，術後2週目17%，薬剤無投与群術後1週目23%であり，共に副腎皮質予備能の高度の低下を認めた。

5. 正常犬群の副腎比体重は0.19g/kgである。脾全剝去犬のEstrogen Insulin併用群では0.18g/kgであり最も正常犬群に近似し，Insulin群の0.17g/kgと共に低値を示したのに反し，Estrogen群では0.23g/kg，薬剤無投与群では0.24g/kgと共に比体重の高値を示した。

6. 脾全剝去犬の副腎皮質の組織所見

Sudan III染色所見では，Estrogen Insulin併用群では長期に亘りSudanoplile granulaの減少を認めず，赤色調に染色された。Insulin群では従来指摘されているように術後1〜2週頃に一時的なSudanoplile granulaの増加を認め，強い赤色調に染色されるが，2週以後はSudanoplile granulaは次第に減少し褐色調に染色される。

Estrogen群では術後Sudanoplile granulaの減少を認め，褐色調に染色される。薬剤無投与群では術後Sudanoplile granulaの減少を認め，褐色調に染色されると共に，術後早期に組織の荒廃が顕著となる。

7. Thorn氏試験に表示される副腎皮質予備能は副腎皮質のSudanoplile granulaと密接な関係にある。即ち脾全剝去犬2週目のInsulin群のThorn氏試験値は60%でありSudanoplile granulaも強い赤色調であり，Estrogen Insulin併用群ではThorn氏試験値は55%でありSudanoplile granulaもInsulin群ほど強い赤色調でない，Estrogen群，薬剤無投与群では副腎皮質予備能の高度の低下を示し褐色調となる。

8. 正常犬の尿中17-KS中に存在する。Androsterone 分画は正常分画ともいえる。このAndrosterone分画値と副腎皮質の赤色調に染色されるSudanoplile granulaとは密接な関係にあると推察される。又脾全剝去犬の術後現出するMethyltestosterone分画は病的分画ともいえるが，このMethyltestosterone分画値と副腎皮質の褐色調に染色されるSudanoplile granulaは密接な関係にあると推察される。

即ち脾全剝去犬の術後2週目の尿中17-KSの醇層クロマトグラフィーでInsulin群のAndrosterone分画値が7.5γはEstrogen Insulin併用群の4.1γより多いが，Sudanoplile granulaの所見でもInsulin群の方がEstrogen Insulin併用群よりも強い赤色調に染色され増量を示している。

一方，薬剤無投与群の術後2週目の17-KSの醇層クロマトグラフィーでMethyltestosterone分画値は6.1γでありEstrogen群の2.7γ，Insulin群の1.8γ，Estrogen Insulin群の2.1γに比して著明な増量を示しSudanoplile granulaも他群に比較して強い褐色調に染色される。

即ち，Sudan III染色所見ではAndrosterone分画値の高い時期副腎皮質のSudanoplile granulaは強い赤色調に染色され，Androsterone分画値の低い時期Sudanoplile granulaは強い赤色調に染色され，Methyltestosterone分画値の高いとときSudanoplile granulaは強い褐色調に染色され，Methyltestosterone分画値の低いときSudanoplile granulaは強い褐色調に染色される傾向にあると推察される。

9. 脾全剝去術後尿中17-KSは正常分画であるAndrosteroneの分泌は軽度の低下の傾向を示し，病的分画であるMethyltestosterone，その他RF 22における分画の分泌は著明に上昇する。これは正常機能低下，病的機能亢進状態にあると言えよう。

以上脾全剝去術後授与したEstrogen，Insulinはその単独授与でもストレス緩和に働くものであり，Estrogen Insulin併用授与では脾全剝の影響は4群中で一番少ない。