inhibits the appearance of spontaneous mammary tumors in mice when given continuously as a supplement to the diet.

Materials and methods. C3H/He female mice maintained in the authors' laboratory were used. After weaning at 23–25 days of age, the control and TPA groups were fed the commercial diet (CA-1: CLEA Japan Inc., Tokyo) only and the same diet supplemented with TPA (Teijin Ltd., Tokyo) in the proportion of 0.5%, respectively, ad libitum throughout their lives. At 70–80 days of age, one female was mated with one male and the concurrent pregnancy was planned until the sixth lactation or mammary tumor appearance. Each female was checked for palpable tumors every 7 days till 16 months of age when all the mice had tumors or died.

Results and discussion. As shown in the Table, mammary tumor incidence was significantly lower in the TPA group than in the control group after 6 months of age, and the rate of increase of tumor incidence was markedly suppressed during 10–13 months in the TPA group. These results clearly indicate that TPA feeding inhibits the spontaneous mammary tumorigenesis in mice. The average tumor ages were 9.9 ± 0.6 (S.E.) and 7.8 ± 0.3 months in the TPA and control groups, respectively, and the difference between groups was statistically highly significant ($P < 0.001$). No difference between groups was observed in the pattern of estrous cycle, in the growth during the virginal stage, in any of the items examined as the indices of reproductive ability (the interval between mating and parturition, the litter size, the average weights of pups on days 0, 12 and 20, the growth rate of pups and the rearing rate) and further in the ages of mice that died without tumors. Intratumor injection of TPA had no effect on the growth of Ehrlich carcinoma (solid form)[6]. The data obtained herein suggest the host-mediated antitumor effect of TPA through its homeostatic action on the body, while the precise mechanism has not yet been understood. The differences between groups of the mammary tumor incidence were smaller after 14 months than before 13 months, although they were still statistically significant ($P < 0.05$). They may be due to the insufficient ability of TPA in maintenance of the homeostasis of the body in these older ages. CAME and MOORE[7] and POTMESIL and GOLDFEDLER[8] reported the direct inhibitory effect of interferon or its inducer on mammary tumorigenesis in mice. The mode of action of TPA on mammary tumorigenesis is probably quite different from that of interferon.

Zusammenfassung. Die Fütterung von Terephthalsäure hemmt die Bildung von spontanen Tumoren der Milchdrüse bei der Maus.

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The Production of Plasminogen Activator During Perfusion of Isolated Rabbit Kidneys

As in the renal venous blood in vivo there is increase in fibrinolytic activity[1], also during perfusion of isolated kidneys plasminogen activator appears in the outflowing fluid from the renal vein[2]. The amount of the activator detected per unit of time can be used for calculating the enzyme liberation rate of the kidney. The value determined does not depend on the perfusion rate but is dependent upon the composition of the perfusing fluid and the preserved metabolism of the organ[3].

These studies were undertaken with the aim of clarifying the effect of the composition of the perfusing fluid and of the preserved metabolism of the organ on the quantity of plasminogen activator liberated as well as on the amount of the activator retained in the organ after perfusion as compared with the level of the enzyme in the kidney determined before examination.

Materials and methods. The kidneys were removed from the rabbits anaesthesized with uretane after previous heparin administration. The isolated organ was placed in a receptacle in 0.9% NaCl at 37 °C. The perfusing fluid,

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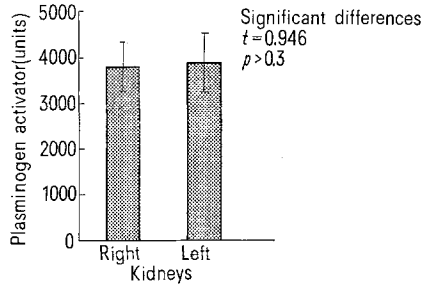

Fig. 1. Plasminogen activator in the 6 pairs of the rabbit kidneys: right kidneys — 342 ± 76 U/g; 3814 ± 520 U/organ; left kidneys — 365 ± 56 U/g; 3887 ± 662 U/organ. Significance differences in activator concentration: $t = 0{,}444$; $p > 0{,}6$.

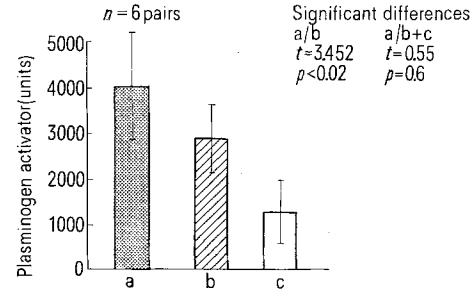

Fig. 2. Plasminogen activator in the kidneys perfused with Tyrode's fluid: a) kidneys before perfusion: 385 ± 63 U/g; 4065 ± 1176 U/organ; b) kidneys after perfusion: 261 ± 59 U/g; 2896 ± 768 U/organ; c) fluid after perfusion: 1306 ± 735 U.

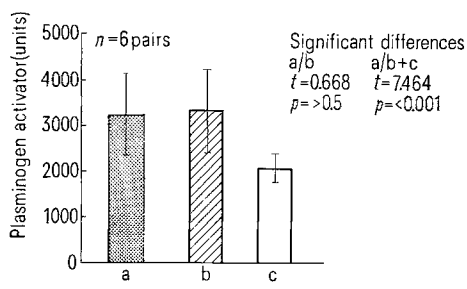

Fig. 3. Plasminogen activator in the kidneys perfused with the 30% vol. oxygenated erythrocyte suspension in Tyrode's fluid: a) kidneys before perfusion: 318 ± 115 U/g; 3235 ± 918 U/organ; b) kidneys after perfusion: 314 ± 119 U/g; 3321 ± 930 U/organ; c) fluid after perfusion: 2071 ± 45_4 U.

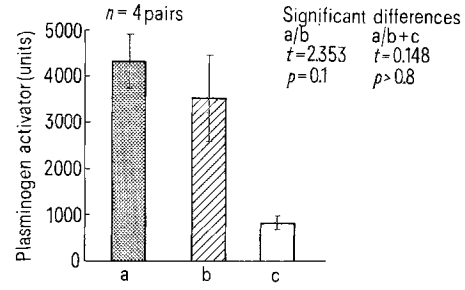

Fig. 4. Plasminogen activator in the NaCN-poisoned kidneys perfused with the 30% vol. oxygenated erythrocyte suspension in Tyrode's solution: a) kidneys before perfusion: 401 ± 57 U/g; 4346 ± 593 U/organ; b) kidneys after perfusion: 318 ± 81 U/g; 3558 ± 965 U/organ; c) fluid after perfusion: 833 ± 149 U.

also at 37 °C, was introduced through a cannula into the renal artery under various pressures, dependent on the perfusion rate needed. The fluid flowed out through a similar cannula introduced into the renal vein.

To accomplish the perfusion either oxygenated Tyrode's fluid without $CaCl_2$ or 30% vol. oxygenated rabbit erythrocyte suspension in the same Tyrode's fluid was used.

In 4 experiments, before the proper perfusion with erythrocyte suspension was performed, the kidneys had been perfused 15 min with 50 ml of 0,9% NaCl containing 2 mg of NaCN.

In order to determine the effect of perfusion on the plasminogen activator content of the kidneys, the organs, after removing the erythrocytes and the capsula, were cut into thin slides and homogenized for 5 min in the presence of Tyrode's fluid in the proportion of 1 g of the tissue – 2 ml of the fluid. Plasminogen activator concentration was determined in the supernatant obtained after centrifuging the material at 2000 *g* for 15 min. The amount of plasminogen activator found in the whole organ's supernatant was also calculated.

Plasminogen activator levels in the fluid perfusing the kidney as well as in tissue extracts were determined in units[4] by the modified euglobulin test:

plasminogen activator in units $= 1300/t - 1300/t_o$

t–fibrinolysis time of the euglobulins obtained from diluted human plasma containing the material examined; t_o–control fibrinolysis time/plasma activator contained in the euglobulins.

The effect of perfusion on the kidney plasminogen activator content was estimated by means of the Student's t-test for series of pairs of measurements: the perfused kidney and the non-perfused one of the same rabbit.

Result and discussion. The comparison of the supernatants of the homogenates of pairs of kidneys of 6 rabbits has revealed that the left kidney and the right one of the same rabbit do not differ significantly, either in relation to the concentration or to the whole amount of plasminogen activator. This estimation allowed us to discover whether the enzyme liberation into the renal vein affects the amount of the activator remaining in the kidney after perfusion as compared with the quantity determined in the non-perfused one.

The amount of plasminogen activator found in the perfusing fluid after 1 h, perfusion of the rabbit kidney with Tyrode's fluid averages 1300 units. The sum of the quantity of the activator found in the perfused kidney's homogenate and detected in the fluid the kidney was perfused with, is equal to the quantity of the activator determined in the second non-perfused kidney of the same animal. The results of the experiments on 6 pairs of the kidneys are shown in Figure 2.

If the perfusion of the kidney is performed with the 30% vol. oxygenated erythrocyte suspension, the amount of the activator yielded over 1 h span is higher than 2000 units. Of particular interest is the that, under these conditions of perfusion, the liberation of significant amounts of the activator into the perfusing fluid does not diminish the quantity of the activator remaining in the organ. This is evidenced by the comparison of the homogenate activator contents of the perfused and non-perfused kidneys. Thus the amount of the activator liberated in 1 h by the kidney perfused with a 30% vol. oxygenated erythrocyte suspension can be regarded as measure of the enzyme production by the isolated organ.

When prior to perfusion with the oxygenated erythrocyte suspension, the kidney was poisoned with NaCN, then, as in the case of perfusion with the Tyrode's fluid, only a decrease in organ activator level is observed. On the other hand, the amount of the enzyme liberated into the renal vein is much lower than in the 2 types of the experiment described above. It should be stressed that the erythrocytes, having flown through the NaCN poisoned organ, did not show any signs of deoxidation.

The result presented here are in favour of the proposition of kidney plasminogen activator production and its liberation into the blood[1,2,5], as well as indicating that the activator production is possible in vitro provided that oxygen supply is secured and organ metabolism maintained[6].

Résumé. On a constaté que la production et la sécrétion de l'activateur du plasminogéne a lieu pendant la perfusion des reins du lapin à l'aide d'une suspension d'érythrocytes oxygenés.

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