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Development of Pulp Stones in Rats with Experimentally Induced Pulmonary Emphysema: A Contribution to the Study of Mechanism of Pulp Stone Formation

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Development of Pulp Stones in Rats with Experimentally Induced Pulmonary Emphysema: A Contribution to the Study of Mechanism of Pulp Stone Formation

Toshiki Tanaka

Abstract

Forty-five male rats of five weeks of age which were operated upon to induce pulmonary emphysema and 51 non-operated rats were examined histologically. The rats which were experimentally induced to develop pulmonary emphysema and whose blood pH was acidic were sacrificed 56 days after the operation. In their molars, pulp stones were found in a considerably high incidence. The pulp stones were examined histologically, histochemically and their hardness compared with that of secondary dentin. They not only resembled those of the human, but also revealed variant stages of development. They were classified into five groups: 1) Pulp stone surrounded by odontoblasts, 2) pulp stone with necrotic pulp cells, 3) pulp stone with degenerated or necrotic epithelial cells, 4) pulp stone with a necrotic blood vessel, and 5) amorphous calcified deposits. The developmental mechanism, the histological and histochemical properties of the pulp stones and of the amorphous calcified deposits were discussed.

Key words: Pulp stone – Pulmonary emphysema – Rat

Introduction

Abnormal calcification of the dental pulp tissue, which is known as denticles, pulp stones or secondary dentin, occasionally gives rise to dental pain[1] or pulp stenosis[2] followed by necrosis. It is also an useful sample for elucidation of the mechanism of calcification of the dental pulp. The developmental process of denticles, however, is still poorly understood for the following reasons: firstly, no precise histological and histochemical studies have been made on a large number of denticles or pulp stones. Secondly, it is practically impossible to obtain a large number of denticles or pulp stones in the variant stages of development.

On the other hand, it is well known that a condition where the blood pH is acidic predisposes to formation of renal calculus, and that denticles or pulp stones are frequently found in extracted teeth of patients of advanced age. It is also known through Csernyei[3] that congested pulp in intact teeth always has an alkaline reaction (pH 7.44) which is higher than that of any other tissue (connective tissue, pH 7.20; blood, pH 7.36).

Up to date, however, there have been only two interesting papers on the experimental study of pulp stone formation excepting the formation of secondary dentin: one by Luostarinen *et al.*[4] and the other by Stenvik and Mjör.[5] The former is a paper on the repair of injured dental pulp by using rats. In that experiment they found large denticles formed in the incisor pulp which was traumatized not only by exposure but also by applying surgical diathermy. The latter is a report on the higher incidence of pulp stones found in human teeth which were extracted after having been subjected to intrusive

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forces by orthodontic treatment.

For these reasons the experiment was commenced with rats which produced metabolic acidosis. This acidic condition was performed in rats affected with experimentally induced pulmonary emphysema. The influences of a low pH in these rats on the formation of pulp stones were studied and then the origin and developmental process of these pulp stones were also studied histologically and histochemically.

Materials and Methods

1. Operation for Pulmonary Emphysema

Tracheal ligatures were made on five weeks old male rats of the Wistar strain according to the method of Ito and Aviado.[6] The trachea was ligated loosely so that stenosis might take place with a suture thread No. 9 around the trachea. On the surrounding tissues special care was taken in order not to damage the thyroid. Similar operations were performed on the control group.

One week after the operation, the following treatments were given for the subsequent four weeks. Ten percent papain (2.5 ml/kg) was perfused into the trachea of the operated rats once a week for the initial two weeks. The same amount of 0.9% physiological saline was perfused into that of the controls. A double amount of papain or physiological saline was perfused into the operated and the control rats respectively once a week for another two weeks.

2. Identification of Induced Pulmonary Emphysema

It was confirmed by the following tests whether the induction of pulmonary emphysema was successful. 1) Functional residual capacity was measured by the method of King.[7] Simultaneously, pulmonary compliance, pulmonary resistance and minute volume were measured according to the method of Dunnill.[8] 2) A histopathological examination was made by counting the ratio of the mean chord length to the internal surface area on the histological preparation with the aid of the point counting method. For the histological preparation, a lung was taken out carefully and fixed with 25 cm/H_2O of 10% neutral formalin solution perfused into the lung via the trachea. A week later, the lung was dehydrated in 80% alcohol solution for 24 hours and in 100% alcohol for 48 hours. Then it was embedded in paraffin. Sections were prepared 8 μ thick and treated with hematoxylin-eosin stain. 3) The pH of arterial blood of the rats affected with pulmonary emphysema was measured by using Corning's 175 automatic blood gas analyser. The pH, PCO_2, PO_2, HCO_3, B.E. (base excess), O_2 content and O_2 SAT (saturation) of the blood were measured simultaneously.

3. Examination of Pulp Stone Development in Operated Rats

The mandibles with molars were removed from the rats. The mandibles and maxillas were immediately placed in isopentane chilled with liquid nitrogen. After freezing, they were freeze-dried at $-40^{\circ}C$ dry temperature for seven days. After being treated with formalin gas, the mandibles were decalcified with 10% EDTA, embedded in paraffin and sliced 4 μ thick with a microtome. The histochemical stains used were hematoxylin-eosin stain, Van-Gieson's stain, Mallory's stain, Masson trichrome stain, periodic acid-Schiff stain, toluidine blue metachromasia (pH 2.5, 4.1, 7.0) and acid hematein stain.

4. Examination of Degree of Calcification of Pulp Stones

In order to study the degree of calcification of pulp stones or calcified tissue, nondecalcified sections were prepared. The mandibles and the maxillas with molars were removed from the rats. Then they were dehydrated in alcohol, and dipped in styrene monomer for 48 hours, and in polyester resin for 48 hours. Then, they were polymerized at $60^{\circ}C$ for 12 hours. The transverse sections of about 40 μ thick were made by sectioning mesiodistally the polymerized mandibular samples using Isomet of Buehler Co.

Contact-microradiograms were made from these sections by using Sofron SRO-M50 of

Soken Co. The photograph was taken under 10 kV, 5 mA for 10 minutes. The obtained spectroscopic safety film 649-0 (Kodak) was sealed with balsam after development, and then enlarged 20 times on A-I film (Fuji). The opacity value obtained from the above-prepared samples by using densitometer PDA-II of Sakura Co. was considered to be the degree of calcification.

Results

1. Identification of Pulmonary Emphysema Induced in Rats

a. Functional examination of the lung

Functional residual capacity (FRC), pulmonary compliance, pulmonary resistance, and minute volume were measured simultaneously. The values of the measurements are shown in Table 1. FRC was 3.05 ± 0.25 ml in the control and 3.15 ± 0.05 ml in the operated group. There seemed to be no significant difference between them. However, in the conversion of FRC/body weight the former was 8.71 ± 0.33 ml/kg, while the latter was 13.25 ± 0.48 ml/kg. This showed a marked difference between them ($p<0.01$).

The value of pulmonary resistance was 0.73 ± 0.038 cm H_2O/ml/sec in the control and 1.450 ± 0.070 cm H_2O/ml/sec in the operated group. The value of the latter was twice as much as that of the control group ($p<0.01$). Therefore, pulmonary compliance of the operated group was apparently smaller than that of the control ($p<0.01$): 0.075 ± 0.005 ml/cm H_2O in the operated group and 0.255 ± 0.012 ml/cm H_2O in the control.

The minute volume of the operated group was also decreased as compared with that of the control group ($p<0.01$): 159.8 ± 33 ml in the operated group and 226.2 ± 5.6 ml in the control.

From these data it was evident that the operated group was afflicted with pulmonary emphysema.

b. Histological examination of the lung

The destruction of pulmonary parenchyma and alveolar wall due to papain was found in the entire lung of the operated group. Therefore, the mean chord length (Lm) was increased and internal surface area (ISA) was decreased in the operated group (Fig. 2).

c. Comparison of acid base balance

The blood pH of the operated group was 7.277 ± 0.106, while that of the control was 7.444 ± 0.041. This meant the blood pH of the pulmonary emphysema rats was more acidic than that of the control ($p<0.01$). As an additional evidence of supportive increase of PCO_2, decrease of HCO_3, and decline of O_2 saturation were recognized in the emphysema rats (Table 2). It is suggested that the acidic blood of emphysema rats was due to respiratory acidosis (Fig. 1).

Table 1

Comparison of Bronchopulmonary Function of the Experimental Emphysema Rats with That of the Control

Group	*Sex and total no. of rats*	*Body weight (kg)*	*FRC (ml)*	*FRC/kg (ml/kg)*	*Pulmonary*		*Minute volume (ml)*
					compliance (ml/cmH_2O)	*resistance (cmH_2O/ml/sec)*	
Control	M (24)	0.35 ± 0.03	3.05 ± 0.25	8.71 ± 0.33	0.225 ± 0.012	0.738 ± 0.038	226.6 ± 5.6
Trachea contraction and papain administration	M (20)	0.24* ± 0.05	3.15 ± 0.05	13.25* ± 0.48	0.075* ± 0.005	1.450* ± 0.070	159.8* ± 3.3

Each value represents the mean ± S.E.
*: Significantly different from the control ($p<0.01$).

2. *Formation of Pulp Stones and Amorphous Calcified Deposits*

a. Incidence and distribution of pulp stones including amorphous calcified deposits

Pulp stones were found in 11 cases out of 51 non-operated rats (22% incidence), whereas there were 27 cases out of 45 emphysema rats (60% incidence) (Table 3). However, as for the number of molars, pulp stones were seen in 22.3% of 268 molars of emphysema rats, and 6.7% of 270 molars of the control rats (Table 4). The distribution of pulp stones was as follows: 45% of the total pulp stones was found in the coronal pulp alone, 24% in the radicular pulp alone and 31% in both of the coronal and radicular pulp.

b. Developmental patterns of pulp stones

The most common occurrence of calcification is the Johnson-Bevelander's so-called early calcification type which is visible in the coronal and radicular pulp. This type is due to degener-

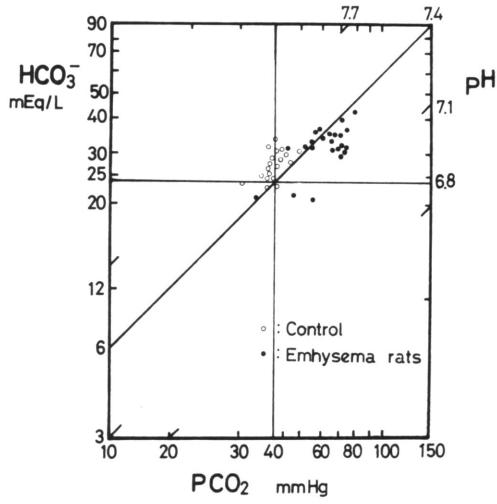


Fig. 1: Relation of PCO_1 and HCO_3 to pH in blood of the experimentally induced emphysema rats.

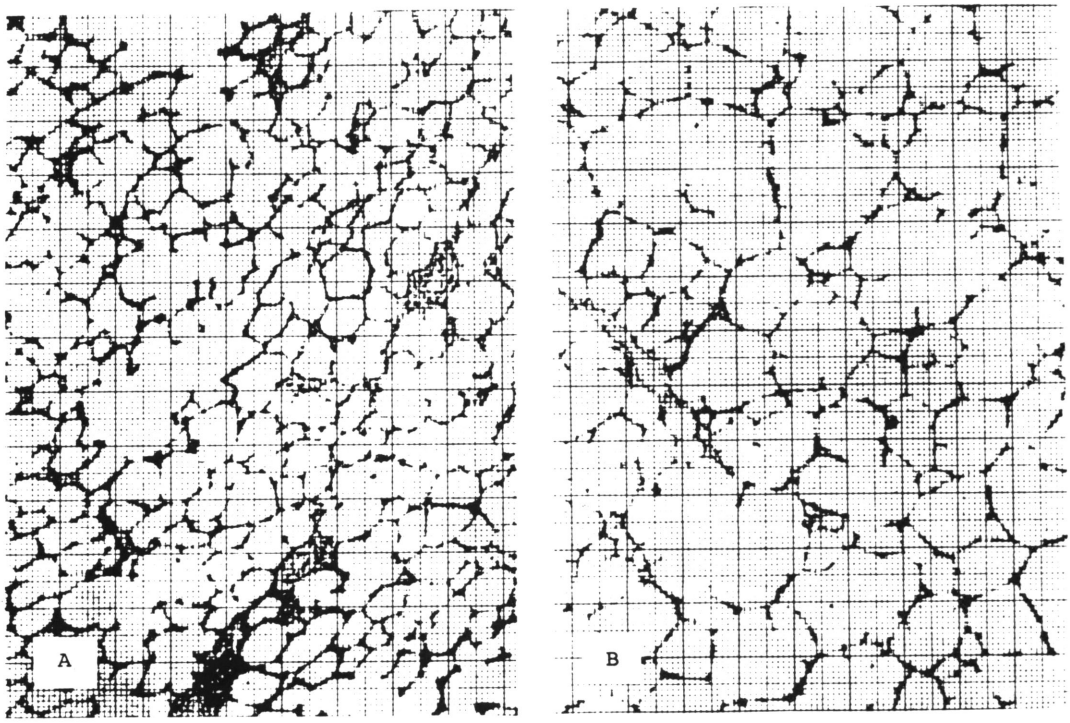


Fig. 2: Section of the lung. H-E stain, A: control rat, B: operated rat with pulmonary emphysema.

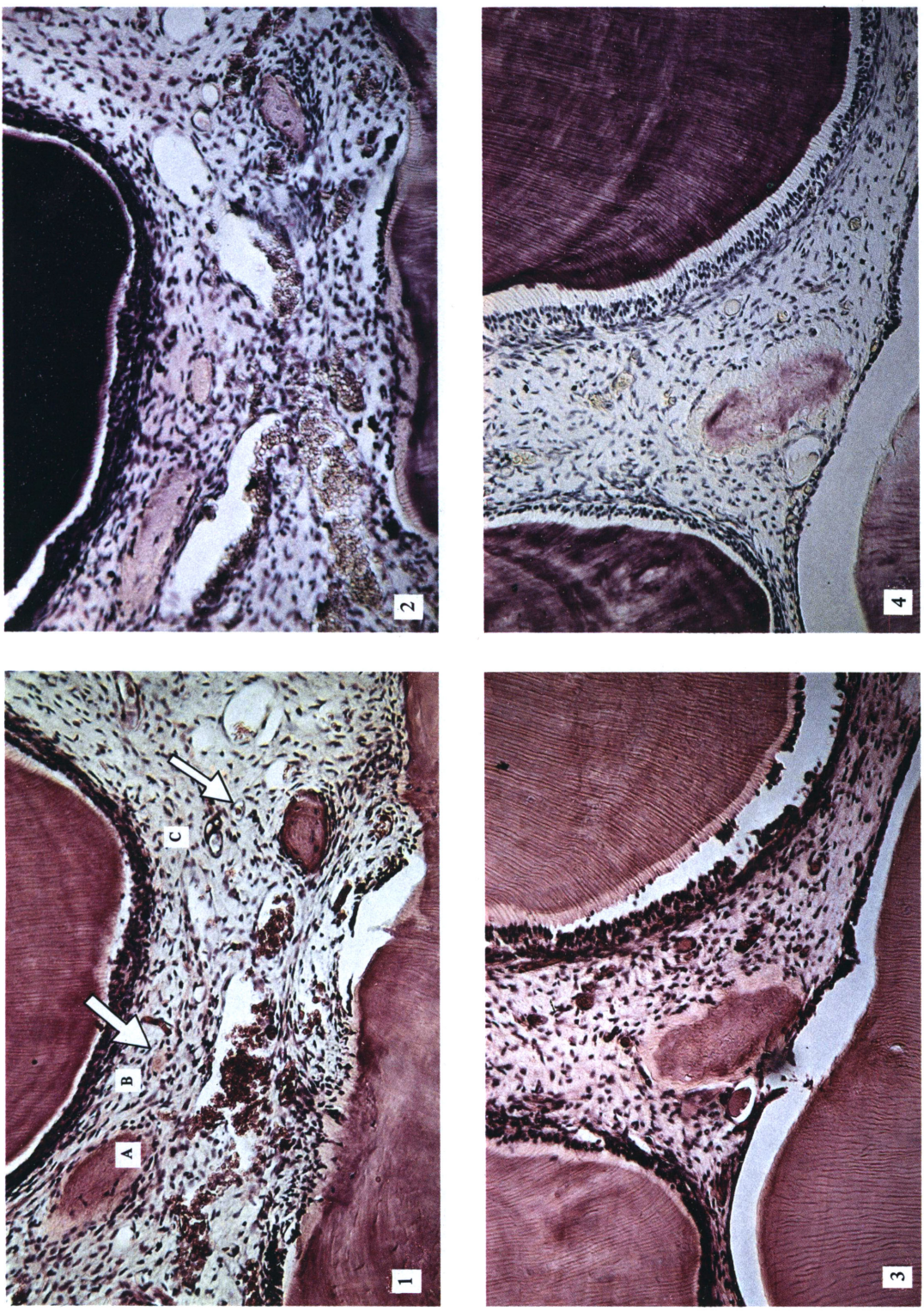
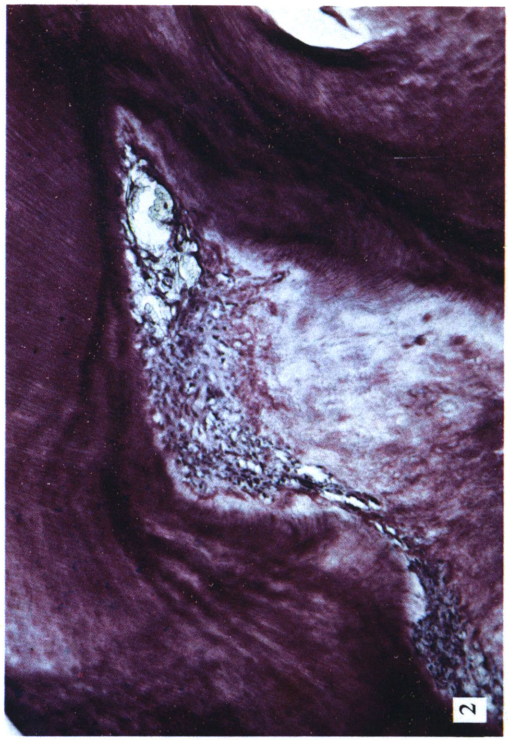
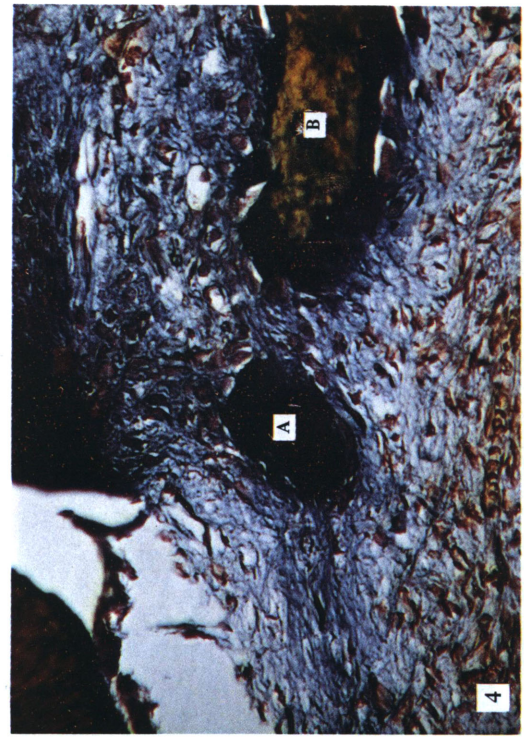
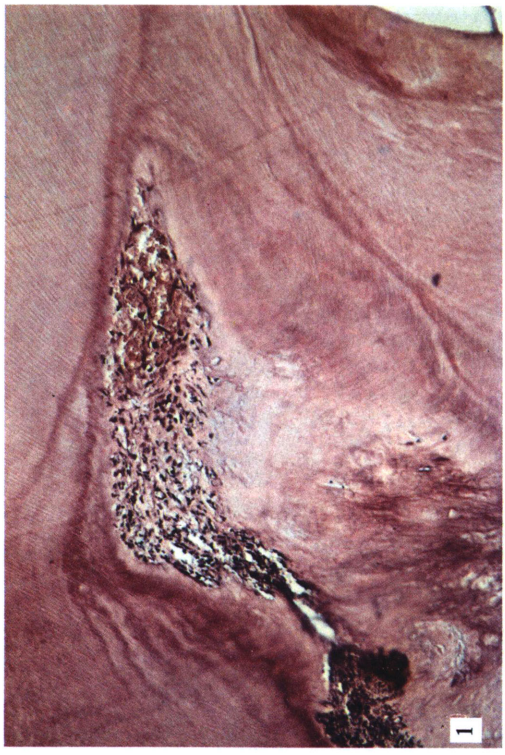


Fig. 3







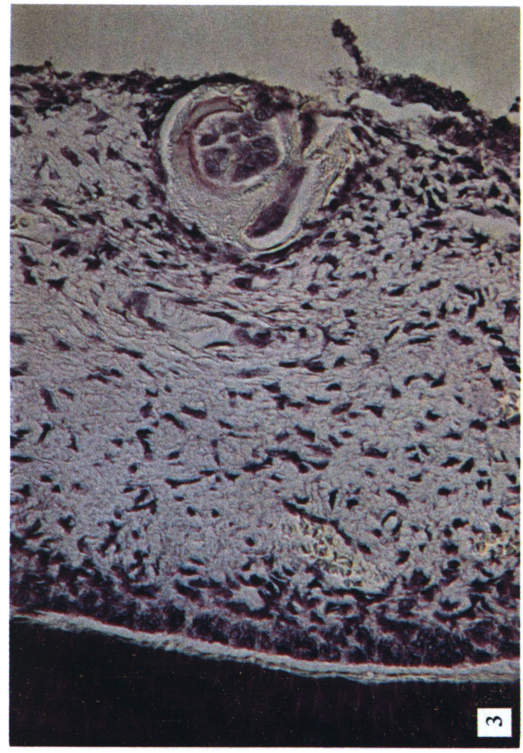


Fig. 4

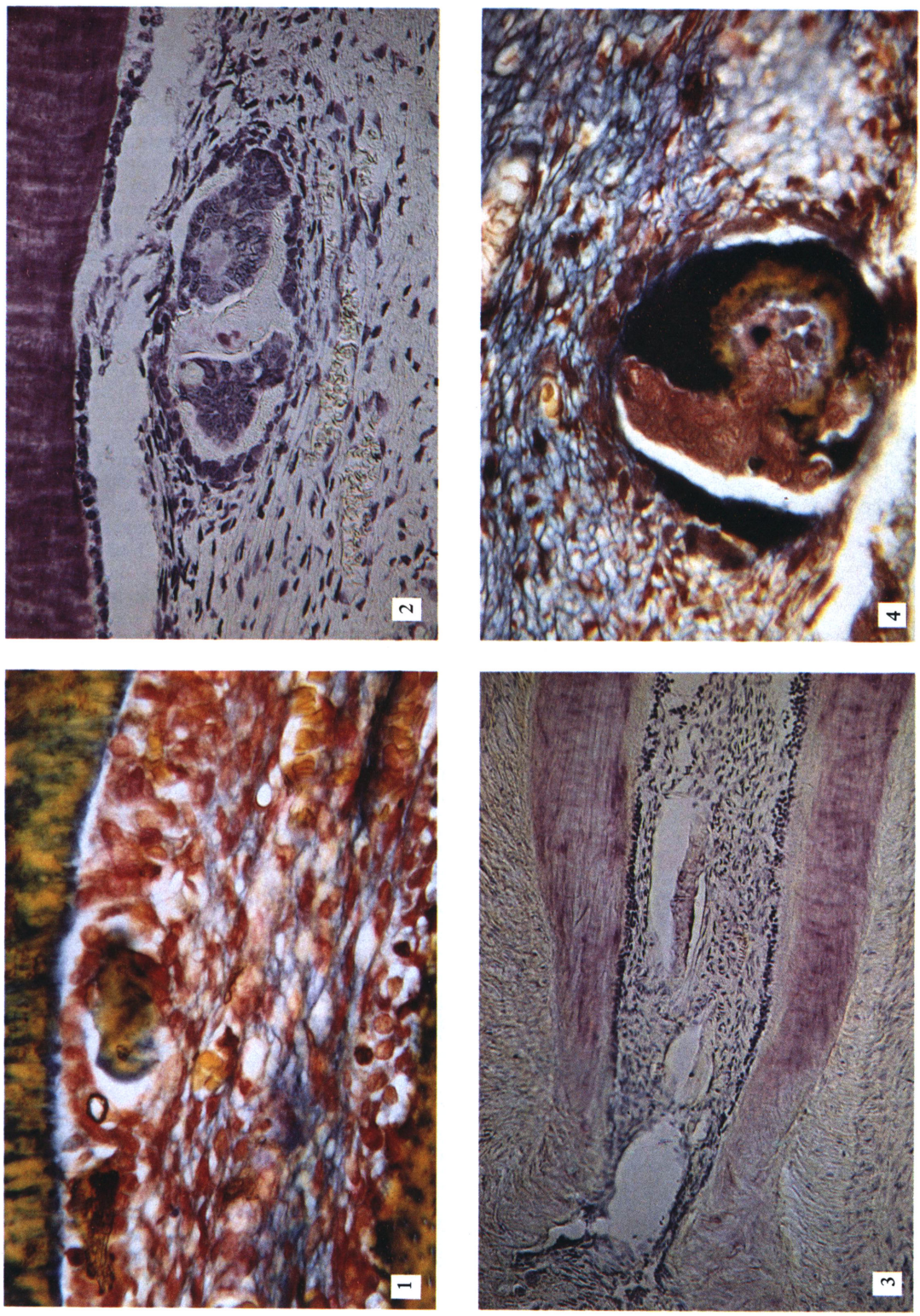
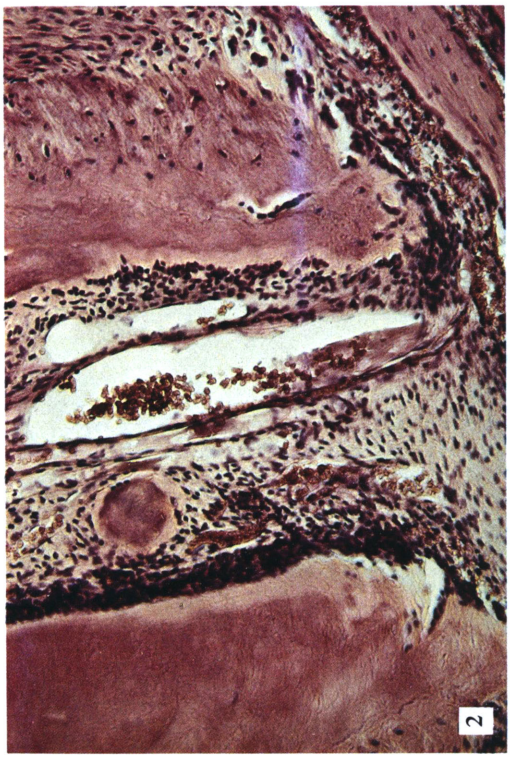


Fig. 5



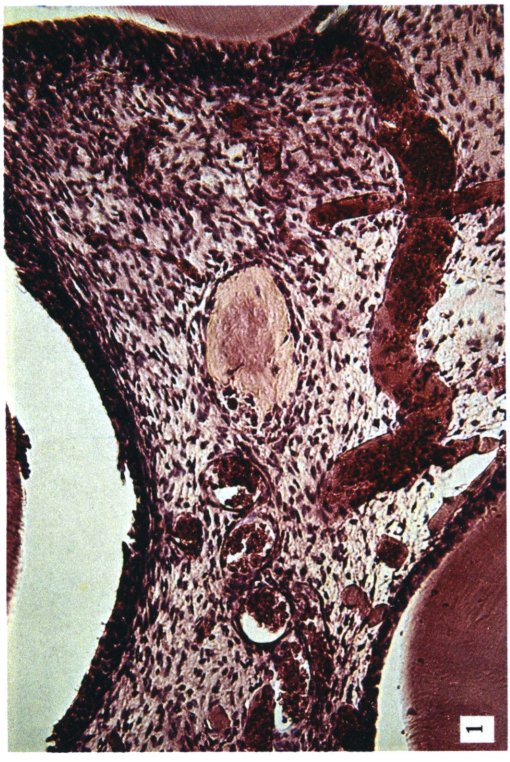


Fig. 6

Explanation of Figures

Fig. 3

1. Coronal pulp of a rat sacrificed 56 days after operation. H-E stain. Pulp stones (A, B) are equivalent to Johnson-Bevelander's so-called early pulpal calcification. Several degenerated or necrotic cells are still visible in A. The nidi are probably pulp cells. No dentinal fibers are recognized. Pulp stone (C) is surrounded by a number of pulp cells. A number of degenerated epithelioid cells are intermingled in a densely packed necrotic cell group of an onion skin-like structure. This one is presumed to be a calcified mass of cells derived from the ectopic epithelial cell rest of Malassez.

2. Adjoining section of the same sample as in Fig. 3-1. Toluidine blue stain (pH 4.1).

3. Coronal pulp of a rat sacrificed 56 days after operation. H-E stain. Pulp stone of a type —early pulpal calcification. Calcification is more advanced in this one than those (A, B) shown in 1. In this section of H-E stain no degenerated cells are visible. However, necrotic cells and cell debris are visible. In H-E stain this pulp stone is identical to a primary dentin although the peripheral part resembles predentin, whereas in toluidine blue stain this one resembles the intermediate of primary dentin and predentin (cf. Fig. 3-4).

4. Adjoining section of the same sample as in Fig. 3-3. Toluidine blue stain (pH 4.1).

Fig. 4

1. Coronal pulp of a rat sacrificed 56 days after operation. H-E stain. Diffuse extensive mineralization was taken place in the coronal pulp. Severely degenerated pulp cells and hemorrhage are visible. This is an unusual case of secondary dentin formation.

2. Adjoining section of the same sample as in Fig. 4-1. Toluidine blue stain (pH 4.1).

3. Radicular pulp of a rat sacrificed 56 days after operation. Toluidine blue stain. In the core of this pulp stone a number of epithelioid cells are visible. These cells are presumed to be ectopic epithelial cell rests of Malassez. This sample is the same as that in Fig. 5-2. The group of cells surrounding the pulp stone are not odontoblasts, but pulp cells such as those found in Fig. 3-2. The core of this pulp stone is not calcified while the peripheral part is well calcified. Therefore, this is a mixed type in which early calcification and multiplying epithelioid cells of Malassez coexist.

4. Coronal pulp of a rat sacrificed 56 days after operation. Mallory stain. The small denatured collagenous mass is so intensely stained that no necrotic cells are visible (A). This could be the prepared locus of calcification for a pulp stone. A pulp stone (B) contains a number of calcified necrotic cells. The peripheral uncalcified part is intensely blue stained.

Fig. 5

1. Radicular pulp of a rat sacrificed 56 days after operation. Some necrotic cells are still visible in the pulp stone.

2. Radicular pulp of a rat sacrificed 56 days after operation. The same sample as shown in Fig. 4-3. Toluidine blue stain (pH 4.1). It is felt that this pulp stone has been fused secondarily, since two groups of epithelioid cells are found. These epithelioid cells are still growing. The cells surrounding the pulp stone are mainly odontoblasts. This could result in a true denticle.

3. Radicular pulp of a rat sacrificed 56 days after operation. Toluidine blue stain (pH 2.5). Two calcified elongated masses are visible. These are derived from the degenerated or necrotic blood vessels.

4. Radicular pulp of a rat sacrificed 56 days after operation. This is a different section from the same sample as in Fig. 4-3. The rosy stained are epithelioid cells which are ectopic epithelial rests of Malassez. Calcified area is stained yellow. Denatured collagenous mass is stained intensively blue. The cells surrounding this pulp stone are mainly odontoblast.

Fig. 6

1. Coronal pulp of a rat sacrificed 56 days after operation. H-E stain. A common type of pulpal calcification. The pulp stone is surrounded by a number of cells. It is different from that with no particular surrounding cells shown in Fig. 3-3. The core of this pulp stone is well calcified as in Fig. 3-3. Some degenerated cells in the less calcified peripheral part of the pulp stone are pulp cells.

2. Radicular pulp of a rat sacrificed 56 days after operation. H-E stain. This pulp stone is well calcified. From the arrangement of the necrotic cells in it, it may have been derived from the ectopic epithelial rest of Malassez.

3. Picture by soft X-ray of the coronal pulp of a rat sacrificed 56 days after operation. The degree of calcification of this pulp stone is slightly less than that of a primary or secondary dentin.

Table 2

Comparison of Acid Base Balance of Emphysema Rats with That of the Control

Group	*Total no. of rats*	*pH*	*PCO_2 (mmHg)*	*PO_2 (mmHg)*	*HCO_3 (mmol/l)*	*B.E. (mmol/l)*	*O_2CT (ml/dl)*	*O_2SAT (%)*
Control	51	7.444 ±0.041	40.9 ±4.1	75.3 ±10.5	28.0 ±2.8	3.7 ±2.6	19.9 ±0.5	94.8 ±2.4
Trachea contraction and papain administration	28	7.277* ±0.106	65.3* ±11.6	65.4 ±15.6	29.5 ±6.9	6.6* ±1.5	18.3 ±2.5	85.6 ±12.3

Each value represents the mean ± S.E.
*: Significantly different from the control ($p < 0.01$).
B.E.: Base excess.

Table 3

Comparison of pH and Incidence of Pulp Stones in Emphysema Rats with Those in the Control

Group	*Total No. of rats*	*Blood pH*	*No. of rats with pulp stones*	*Blood pH*	*Incidence*
Control	51	7.444 ±0.041	11	7.456 ±0.023	22%
Experimental	45	7.277 ±0.106	27	7.277 ±0.112	60%

pH value represents the mean ± S.E.

ation and necrosis of parts of the pulp tissue.

Therefore, some degenerated pulp cells were visible in the pulp stones at an earlier stage of development in this study (Figs. 3-1-A, 5-1 and 6-1). As calcification advanced, degenerated pulp cells became necrotic and obscure (Figs. 3-1-A and 6-1). The central part of the pulp stones of this type was generally more calcified than the periphery (Figs. 3-3 and 6-1). Two different aspects were seen in pulp stones of this type: one was surrounded by a number of pulp cells (Fig. 6-1) while the other was not surrounded by any particular group of cells (Figs. 3-1-B and 6-2).

In case where ectopic epithelial cells of Malassez were involved in calcification, two different types of pulp stones appeared. One was a type of false denticles, the nidi of which were the degenerated epithelial cells. These epithelial cells become elongated in large number. Later the center of the epithelial bar underwent degeneration and became necrotic to be calcified and finally resulted in pulp stones (Figs. 5-2 and 6-2). The other was a type of true denticles. Some of the epithelial cells of the ectopic group formed a cyst, and the remaining group of epithelial cells induced the surrounding pulp cells to differentiate into odontoblasts. Under these conditions, if the epithelial cells forming a cyst underwent degeneration, cell proliferation ceased and calcification commenced. Then the degenerated cells were embedded as nidi and newly formed odontoblasts produced true denticle formation

in cooperation with some pulp cells. The developmental process of this type of denticles are shown in Figs. 4-3 and 5-4.

Degenerated fragmental blood vessels were often found in the operated group. In most of the cases no pulp cells were found around the vessels forming a vacuole or tissue defect, and vessels tended to be necrotic and eventually were calcified as a whole. Formation of such a type of pulp stone, the nidi of which were fragmental blood vessels, is shown in Fig. 5-3.

Diffuse calcification occurred occasionally in an extensive area of the pulp tissue (Figs. 4-1 and 4-2). This type of calcification resembled an unusual case of secondary dentin formation.

In the present study, no diffuse calcification was found which took place in the radicular pulp embedding a number of blood vessels.

Based upon these findings, pulp stones including amorphous calcified deposits can be classified as follows:

1. Pulp stone surrounded by odontoblasts—Dentinal fibers were seen and the nidi were presumed to be Malassez's rest epithelial cells which were ectopic. This type was identical with the true denticle.

2. Pulp stone partially surrounded by pulp cells—The nidi were necrotic pulp cells. No dentinal fibers were seen. These were a type of false denticles.

3. Pulp stone totally surrounded by pulp cells—The nidi were a large number of degenerated or necrotic epithelial cells.

4. Pulp stone exhibiting mineralized necrotic blood or lymph vessel (Some cases contained a number of necrotic erythrocytes)—In the present study no calcification was seen in the surrounding vessels which remained completely functional.

5. Amorphous calcified deposits—In cases where diffuse calcification occurred minimally one involved a number of blood vessels within the radicular pulp while others occurred outside of the blood vessels without their degeneration per se.

The results of histochemical reactions on the pulp stones in the rats are shown in Table 6.

Table 4

Distribution of Pulp Stones in Molars of Emphysema Rats and Control Rats

Group	*Total no. of molars*	*No. of molars with pulp stones*	*Coronal pulp only*	*Radicular pulp only*	*Both coronal and radicular pulp*
Control	270	18	10 (56%)	6 (33%)	2 (11%)
Experimental	268	60	25 (42%)	13 (22%)	22 (36%)
Total	538	78	35 (45%)	19 (24%)	24 (31%)

Table 5

Comparison of Radiopacity of the Pulp Stone with That of the Other Dental Tissues

	Pulp	*Enamel*	*Primary Dentin*	*Secondary Dentin*	*Pulp Stone*
Value of radiopacity	0.34 ±0.19	0.95 ±0.02	0.78 ±0.08	0.73 ±0.03	0.68 ±0.13

Each value represents the mean ± S.E.

c. Calcified density of pulp stone

Calcified density of the pulp stone was compared with that of other dental tissues such as the secondary dentin and the primary dentin (Table 5). The density of the pulp stone was less than that of the primary dentin or secondary dentin. The pulp stone showed higher density value in the central part than the peripheral part (Fig. 6-3). Pulp stones showing lower radioopacity than the primary dentin or the secondary dentin were not observed in this study.

Discussion

1. Relationship between pH Value of Dental Pulp and That of Blood

Our data on pulmonary emphysema was induced in all rats and, therefore, they served the purpose of our experiments. According to

Table 6

Histochemical Reactions on the Pulp Stones in the Operated Rats

Staining methods	*Type 1*		*Type 2*		*Type 3*		*Type 4*		*Type 5* *A*		*Type 5* *B*	
	I	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*	*I*	*II*
H-E	++	++	+	++	++	++	++	+	+	+	+	+
Van Gieson	++	+	++	+	+	+	++	+	+	+	+	+
Mallory	++	+	++	+	+	+	±	+	+	+	+	+
Masson trichrome	++	+	++	+	+	+	++	+	+	+	+	+
Periodic acid schiff	+	+	+	+	+	+	++	+	+	+	+	+
Toluidine blue pH 7.0	++	++	+	++	++	++	++	+	++	++	++	++
pH 4.1	++	++	±	++	++	++	++	+	++	++	++	++
pH 2.5	+	+	–	+	+	+	+	±	+	+	±	±

I: periphery of pulp stone, II: center of pulp stone, A: tertiary dentin, B: amorphous calcified deposit.

Table 7

Histochemical Reactions on the Pulp Stone in the Humans

Staining methods	*True denticle*		*False denticle*		*A*		*B*	
	I	*II*	*I*	*II*	*I*	*II*	*I*	*II*
H-E	+	+	++	+	++	++	++	++
Van Gienson	++	++	++	++	+	+	+	+
P.A.S.	++	++	++	++	++	++	++	++
Alcian blue	±	±	++	+	±	±	+	+
Van Kossa	++	++	++	++	++	++	++	++
Alizarin red S	++	+	++	+	++?	+?	++?	+?

Source: Nakamura *et al.*[18]

I: peripheral parts of calcified body, II: center of calcified body, A: calcareous degeneration, accompanied with hyaline degeneration, B: calcareous degeneration, not accompanied with hyaline degeneration.

Fisher[13] the dental pulp is a little alkaline (pH 7.44) and Ca^{2+} is transferred from the dentin and pulpal fluid if the blood is acidic. This means the pH value of the pulp is always kept constant. Therefore, the more acidic the pH of the blood becomes, the more Ca^{2+} is transferred from these sources. Under such a condition the Ca^{2+} concentration of the pulp is presumed to become unusually higher. This might be one of the major factors for bringing about a higher incidence of pulp stones in rats with pulmonary emphysema. The blood pH of these animals was lower than that of the controls and the incidence of pulp stones in these operated rats was 60%, while that of the controls was 22%. Another reason for production of pulp stones is presumed to be inflammation of the pulp. It is felt that inflammation not only makes the pulpal fluid acidic but also brings about malcirculation of the blood in the pulp accompanied by initial generation of some pulp cells. The latter could give rise to not only secondary degeneration of the pulp cells but also partial necrosis of the blood vessels. These interpretations would go well with the fact that the incidence of pulp stones is 90% in persons of 50 to 60 years of age while it is 66% in younger persons of 10 to 20 years of age (Hill[14]).

The value of 66% for incidence of pulp stones in young persons seems to be reasonable compared with that of 56% for incidence of pulp stones in permanent teeth extracted for orthodontic treatment (James[15]). The pulp stone of low pH rats seemed to be the same as those shown in Figs. 1-1 and 3-4. Since the density of calcification of the pulp stone was higher in the central part than in the peripheral part, it was considered that they were in an earlier stage of development.

2. Calcification of Pulp Stones

Most of the studies on pulp stones by previous workers have been mainly based upon the ground or decalcified sections which were obtained from a large number of extracted teeth with caries or parodontosis, and partly based upon those of a smaller number of extracted teeth of young persons with no caries for orthodontic treatment and those of the smallest number of cadavers. Therefore, much is known about matured pulp stones in teeth extracted from persons of advanced age. However, there has been relatively little advance in the property of pulp stones which appeared in the caries-free extracted teeth of the younger persons and little is known about the developmental process of pulp stones.

According to location, pulp stones or denticles are classified as follows: free, attached and embedded, or interstitial. Their structures are classified as follows: true, false and calcareous degeneration (i.e. diffuse or amorphous) (Orban,[9] Sundell *et al.*,[10] Ishikawa and Akiyoshi[11]).

The structure of a true denticle is similar to that of primary dentin because it exhibits dentinal tubules containing the process of odontoblasts surrounding their surface. They are believed to be caused by remnants of epithelial root sheath or, strictly speaking, formed by secondarily differentiated odontoblasts which are derived from pulp cells owing to epithelial rest cells of Malassez.

On the other hand, the structure of false denticles is variant. The structure does not exhibit dentinal tubules but appears as concentric layers of calcified tissue. According to Orban[9] the structure is of three types: 1) False denticles appear within a bundle of collagen fibers combined with necrotic cells; 2) They appear in a location in the pulp free of collagen accumulation, although they contain a number of necrotic or calcified cells; 3) They arise around either lymph vessels or blood vessels, as shown in Fig. 5-16 in Orban's book.[9] The electron photomicrograph indicates the cross section of lymph vessels. Therefore this type of false denticles seems to resemble the diffuse calcification along the vessels; 4) They arise as calcified *throumbi* in blood vessels, which may also serve as nidi for false denticles.

Pulp stones, in a broader sense, i.e. diffuse calcification or calcareous degeneration, appear as irregular calcific deposits in the pulp tissue, following collagenous fiber bundles or blood vessels. This mineralized deposit is stained intensely with hematoxylin, but stainability of

the matrix is entirely different from that of dentin. This is usually located in the root canal, less often in the coronal area.

The great majority of pulp stones reported by previous workers represented a matured state of calcification. It is felt that a smaller number of immature pulp stones and developing abnormal calcification of the pulp tissues have been overlooked even if they actually exist.

The pulp stones including abnormal amorphous calcified deposits, in a broader sense, were found in molars of the rats with pulmonary emphysema. These pulp stones were variant not only in matured shape, but also in the developmental stage. According to the author's classification mentioned above, interpretation of the development of the different types of pulp stones can be made as follows:

1. A pulp stone surrounded by odontoblasts, the nidi of which are presumed to be the epithelial rest cells of Malassez – This is a true denticle in which dentinal fibers are seen. The property of this pulp stone closely resembles that of the primary dentin. The nidi of this pulp stone are probably the epithelial rest cells of Malassez which are ectopic. This interpretation is based on the assumption that odontoblasts surrounding the surface of pulp stones are derived from pulp cells which are induced (in the beginning) to become odontoblasts by these particular epithelial cells. The epithelial cells of Malassez are ectopic disintegrated epithelial sheaths of Hertwing which were originally integrated with the inner dental epithelium.

2. A pulp stone, the nidi of which are necrotic pulp cells – This is a common type of false denticle. During the course of development of this pulp stone formation two different aspects are seen: one is surrounded by a number of pulp cells while the other is surrounded by none. The latter case represents the state of pulp cells soon after or a little while after the pulp tissue is broken down, whereas the former indicates the state of forming or having formed a pulp stone.

3. A pulp stone, the nidi of which are degenerated or necrotic epithelial cells – These epithelial cells are presumed to be the ectopic epithelial rest cells of Malassez. These epithelial cells are, if not ectopic, usually proliferate and develop into epithelial pearls. However, if they move into the pulp, they undergo degeneration, become calcified during the course of development, and fail to become an epithelial pearl. For this reason, no dentinal fibers are seen in this type of pulp stone. It is another type of false denticle. This type was reported by Orban[9] (Fig. 5-18: B of his book), although he made no comments of its genesis.

4. A pulp stone, the nidi of which is a necrotic blood vessel – As for pulp stones exhibiting a mineralized blood vessel, most of the cases are mineralized lumina of vessels and some are entirely calcified thrombosed vessels. Calcification surrounding the vessels which remained completely functional was reported by Sundell *et al.*[10] (Figs. 5 and 6 their paper).

5. Amorphous calcified deposits – In case where a small amount of the pulp tissue undergoes degeneration to become calcified, a small pulp stone is brought about, which is identical with Johnson-Bevelander's[12] so-called early calcification of the pulp tissue. However, in case where an extensive area of the pulp tissue undergoes degeneration to become calcified, diffuse extensive mineralization takes place, which is an unusual case of secondary dentin formation.

In another case where diffuse calcification takes place in the radicular pulp, a number of blood vessels are involved or diffuse calcification takes place only at the outside of a blood vessel. In the latter case the vessel itself is not degenerated. This type of calcification was reported by Orban[9] (Fig. 5-16 of his text).

3. Comparison of Incidence of Pulp Stones of Rats

The incidence of pulp stones was 22% in the control rats, whereas it was 60% in the operated rats. These numbers were obtained by counting the number of rats in which pulp stones were found. According to the number of molars in which pulp stones were found, the incidence of pulp stones was 6.7% in the 270 molars of control rats whereas it was 22.3% in the 268 molars of operated rats.

According to Thomas[16] the incidence of

pulp stones in the caries-free third molars is 27%. On the other hand, that of the inflammation-free pulp is 6% in the report by Hall.[17] These human data were obtained by counting the extracted teeth. The data indicating 6.7% in the control rats with no caries may agree to certain degree with the above-mentioned data of the human teeth with no caries. At this moment, however, it seems to be difficult to put an appropriate interpretation on the relatively high incidence of pulp stones in the caries-free molars of non-operated young rats.

Histochemical comparison of pulp stones in rats with pulmonary emphysema with those of the human is shown in Table 7,[18] which indicates that pulp stones of the rats also resemble those of the human in the histochemical aspect.

Conclusion

So far as examined by previous workers, the great majority of pulp stones were obtained from extracted teeth with caries or parodontosis and they represented matured states of calcification. Although, the pulp stones obtained from caries-free extracted teeth were small in number, they also represented a matured state of calcification. No study has been made on variant developmental stages of pulp stones.

The rats whose blood pH were acidic were experimentally produced. In these rats a considerably high incidence of pulp stones was found. Pulp stones which were brought about indicated not only the variant shapes and variant developmental stages but also the similarity to those of humans in many respects. A histological study was made by the author on the variant developmental stages of these pulp stones and it was suggested that when the blood was acidic, parts of the pulp became more alkaline, collecting Ca^{2+} from the dentin and pulpal fluid and pulp stones were easy to develop under such a condition. As for elucidation of the developmental mechanism of pulp stones in rats with acidic blood, however, a further study on the pH of the pulp will be necessary.

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PROSTAGLANDIN E_2 AND CYCLIC NUCLEOTIDES DURING ANAPHYLACTIC SHOCK IN RATS*

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Abstract—Anaphylactic shock was induced with ovalbumin in sensitized rats and the relationship between PGE_2 and cyclic nucleotides in lung tissue and plasma histamine during anaphylactic shock was studied. PGE_2 level and cyclic AMP/cyclic GMP ratio decreased with this ovalbumin-challenge, and the former reached a minimum value 40 sec after the challenge while the latter reached a minimum value 20 sec later. The plasma histamine level was elevated and reached a maximum value concomitant with the minimum value in the cyclic AMP/cyclic GMP ratio. Dibutyryl cyclic AMP elevated the PGE_2 level significantly and inhibited the ovalbumin-induced elevation of plasma histamine, however, this effect was abolished by the administration of indomethacin. PGE_2 infusion elevated the cyclic AMP level as well as the cyclic AMP/cyclic GMP ratio, in a time-dependent manner, and inhibited the ovalbumin-induced elevation of plasma histamine during 10 min infusion. There was a significant correlation between the cyclic AMP level and the cyclic AMP/cyclic GMP ratio, both elevated by PGE_2 infusion. Thus, anaphylactic elevation of the plasma histamine level results from a decrease in the levels of PGE_2 in lung tissue rather than a decrease in the cyclic AMP/cyclic GMP ratio, albeit these decreases being coincident during anaphylactic shock.

Acute anaphylactic shock is a systemic allergic reaction which occurs in an appropriately sensitized individual following re-exposure to the challenging antigen. When the antigen contacts mast cells coated with immunoglobulin, IgE, the mast cells are degranulated and large amounts of histamine and slow reacting substance of anaphylaxis (SRS-A) are released (1, 2). Histamine induces a contraction of smooth muscle and dilatation of capillary beds. The most detrimental effects of histamine action in anaphylaxis are the constriction of bronchioles and bronchi and peripheral vasodilation after which there is a rapid fall in blood pressure (3).

Histamine release is inhibited by theophylline (4–6) in rats (4, 5) and this effect is dependent on adenosine-3′,5′-cyclic monophosphate (cyclic AMP) (5, 7) which is known to play a role in the functional regulation of many organs (8). In a previous study, changes in cyclic AMP levels during anaphylactic shock were found to be dependent on prostaglandin (PG) E_2 levels in lung tissue (4), such being considered a target organ of systemic allergic reaction in guinea-pigs (9–11). We suggested that the onset of anaphylactic

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shock may possibly result from a decrease in PGE_2 levels rather than a decrease in cyclic AMP levels in lung tissue. Recently, we reported that during anaphylactic shock, histamine release was promoted by agents that lower concentrations of cyclic AMP or elevate levels of guanosine-3′,5′-cyclic monophosphate (cyclic GMP) (12). The cellular expression of extracellular stimuli by a bimodal action of cyclic nucleotides has also been suggested (13).

Since PGE_2 and cyclic nucleotides were thus assumed to be involved in onset of anaphylactic shock, in the present study we investigated the relationship between cyclic AMP/cyclic GMP ratio and PGE_2 content in lung tissue, and attempted to determine whether the onset of anaphylactic shock was due to changes in the cyclic AMP/cyclic GMP ratio or to changes in levels of PGE_2.

MATERIALS AND METHODS

Male Wistar rats 4 weeks of age underwent one week of acclimatization after purchase, then 0.5 ml of a 2% ovalbumin-complete adjuvant emulsion per rat was then given i.m. into the hind-leg twice a week for 5 weeks. After the sensitization, the antibody titre of IgG was about 64-fold and that of IgE was 43-fold. All experiments were carried out in sensitized rats anesthetized with sodium pentobarbital (30 mg/kg, i.p.). Anaphylactic shock was induced by i.v. administration of 0.2 ml of 2% ovalbumin-physiologic saline per rat.

Cyclic nucleotides and PGE_2 were assayed in the inferior lobe of the dextral lung.

Determination of cyclic nucleotides

Experimental animals were decapitated, the whole lung was excised and immediately fixed by focussed microwave irradiation (600W, 2,450 MHz) for 5 sec (14). The blood was washed out by physiologic saline via the pulmonary artery and the physiologic saline was removed by blotting paper. Each sample was prepared into 100 mg wet weight.

Cyclic AMP: For extraction of cyclic AMP, 1.0 ml of 6% (W/V) trichloroacetic acid (TCA) was added to the samples, and the mixture was homogenized. After centrifugation at 3,000×g for 20 min and removal of a protein fraction, 0.1 ml of 1N-HCl was added. Extraction with a 2-fold volume of ethyl ether was repeated five times to remove TCA, then the remaining supernatant was warmed to 80°C in a warm bath in a draft, to completely evaporate the remaining ether. The liquid phase was lyophilized and was redissolved in 2.0 ml of a 50 mM sodium acetate buffer (pH 4.0), and this sample was preserved at −20°C. The quantitative determination of cyclic AMP was carried out by the protein binding method of Gilman (15).

Cyclic GMP: Cyclic GMP was extracted from the homogenates and purified by ion-exchange chromatography (16, 17). The tissue homogenate, containing 6% (W/V) TCA, was centrifuged at 27,000×g for 30 min 50 μl of 4N-HCl were added to 4.0 ml of the supernatant, and this solution was extracted three times with 5.0 ml of ethyl ether. Two ml of the water phase was lyophilized, redissolved in 1.0 ml of 50 mM sodium acetate buffer (pH 4.0), and applied to a Dowex AG1-X8 column (0.5×5 cm) equilibrated with distilled

water. The column was washed with 10.0 ml of water and 10.0 ml of 2N-formic acid before the cyclic GMP was eluted with 14.0 ml of 4N-formic acid. The column eluate was lyophilized and redissolved in 0.5 ml of 10 mM sodium acetate buffer (pH 4.0) for determination of cyclic GMP concentration. Cyclic GMP was assayed according to the method of Illiano et al. (18).

All determinations of cyclic nucleotides were performed in duplicate.

Determination of plasma histamine

Plasma histamine levels were measured by a modified method of Shore et al. (19). Blood taken from the pulmonary artery was immediately centrifuged at 10,000 r.p.m. at 4°C for 10 min and plasma was obtained; a 9-fold volume of 0.4N-$HClO_4$ was added to the plasma obtained, and the mixture was centrifuged at 3,000 r.p.m. at 4°C for 15 min. A protein fraction was then removed and histamine was extracted with butanol from the supernatants. After the extraction, histamine was coupled with O-phthalaldehyde (OPT) at a highly alkaline pH, and the fluorescent assay was conducted. The OPT from commercial source (Sigma Chemical Co.) was purified (20, 21) for the assay. Histamine fluorophor was read in a spectrofluorometer (Hitachi, 204) at the following wavelengths: activation, 360 mμ; fluorescence, 450 mμ.

Determination of PGE_2

For the determination of PGE_2, 200 mg wet weight of lung tissue was weighed and immediately 10-fold volume of 10^{-3} M-indomethacin/L-95% ethanol was added. Homogenization in a water bath kept at 0°C using a cooling circulator (Komatsu-Yamato, CTR-120) followed. Precipitates were allowed to remain overnight at 4°C (22) and were then centrifuged at 3,000×g for 15 min. Each precipitate was washed with absolute ethanol three times and the supernatants were combined and evaporated to near dryness. The residues were redissolved in ethanol-water (2 : 1, by volume) and washed three times with petroleum ether (boiling point: 40–60°C). After removal of the petroleum ether phase, the ethanol was removed with a concentrator (Taiyo, TX-8) before acidification to pH 3.0 with 1N-HCl. The aqueous phase was then extracted three times with equal volumes of ethyl ether. The organic phases were combined, evaporated to dryness, and redissolved in acetatemethanol (3 : 1, by volume). Total PGs were separated into PGE_1, PGE_2, $F_{1\alpha}$, and $F_{2\alpha}$ by the stepwise development method (23) applied to thin-layer chromatography (TLC) on 5% (W/V) silver nitrate-sprayed silicagel HR plate after first development to separate the PGsE and PGsF. Solvent used in this determination; first step was chloroform—ethyl acetate—ethyl alcohol—acetic acid (200 : 200 : 7.5 : 10, V/V), the second step being the same after the silver nitrate was sprayed.

The determination of PGE_2 was performed by the radioimmunoassay of a double antibody method according to the method of Levine et al. (24). In our method, final recovery of PGE_2 was usually about 96%.

Drugs and treatments: Dibutyryl cyclic AMP sodium (P-L Biochemicals Co.); cyclic AMP assay kit, cyclic GMP assay kit (Boehringer Mannheim Co.); prostaglandin radio-

immunoassay kit (Clinical Assay Inc.); indomethacin (Sumitomo Kagaku Co.). Prostaglandin E_2 was a gift from Japan Upjohn Co.. Other reagents were of analytical grade from commercial sources.

Dibutyryl cyclic AMP and indomethacin were administered in a volume of 0.1 ml/100 g body weight. Dibutyryl cyclic AMP was given (5 mg/kg, i.v.) 10 min before ovalbumin-challenge. Indomethacin was given (10 mg/kg, i.p.) 15 min before ovalbumin-challenge or dibutyryl cyclic AMP administration. PGE_2 was infused (0.5 μg/50 μl/kg/min) via the femoral vein.

RESULTS

Interrelation of cyclic AMP/cyclic GMP ratio, PGE_2 and histamine during anaphylactic shock: Ovalbumin-induced changes in the cyclic AMP/cyclic GMP ratio and PGE_2 level in lung tissue and plasma histamine level in sensitized rats are depicted in Fig. 1.

A statistically significant decrease ($P<0.05$) in cyclic AMP was observed 20 sec after ovalbumin-challenge, and the cyclic AMP level continued to decrease. The cyclic GMP

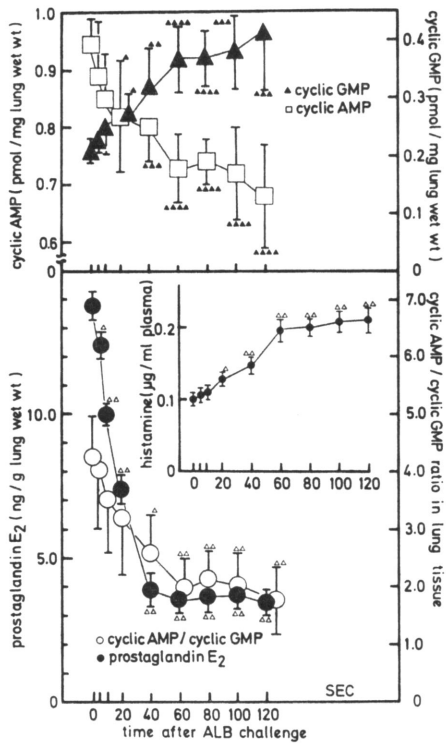

FIG. 1. Changes of cyclic AMP/cyclic GMP ratio, PGE_2 content in lung tissue and plasma histamine content during anaphylactic shock induced with ovalbumin. Each point represents the mean±S.D. from 5 experiments. ALB=2% ovalbumin 0.2 ml/rat i.v. Significance of difference from zero-time value studied by Student's *t*-test $^{\triangle}p<0.01$; $^{\triangle\triangle}p<0.001$, Aspin-Welch method $^{\blacktriangle}p<0.05$; $^{\blacktriangle\blacktriangle}p<0.02$; $^{\blacktriangle\blacktriangle\blacktriangle}p<0.01$; $^{\blacktriangle\blacktriangle\blacktriangle\blacktriangle}p<0.001$.

level began to increase after the ovalbumin-challenge and the increase was significant ($P<0.05$) 20 sec after the challenge. Rapid decreases in the cyclic AMP/cyclic GMP ratio and PGE_2 levels were seen after the ovalbumin-challenge, the former reaching a minimum value in 60 sec and the latter in 40 sec. Regarding the plasma histamine level, a statistically significant increase ($P<0.01$) was seen 20 sec after ovalbumin-challenge and the plasma histamine level reached the maximum value 60 sec after the challenge.

Effect of PGE_2 on cyclic AMP/cyclic GMP ratio: Effects of PGE_2 infusion from zero to 20 min on cyclic nucleotides level in lung tissue are depicted in Fig. 2.

Cyclic AMP levels were elevated in a time-dependent manner, and there was a significant elevation ($P<0.01$) after a 5 min infusion. The cyclic GMP level was decreased significantly ($P<0.001$) during the 15 min infusion. The cyclic AMP/cyclic GMP ratio was elevated in a time-dependent manner, and a significantly high value ($P<0.01$) was observed after a 5 min infusion. There was a significant correlation ($r=0.912$, $P<0.01$) between changes in levels of cyclic AMP and changes in the cyclic AMP/cyclic GMP ratio, as induced by PGE_2 infusion (Fig. 3).

Correlation of changes in the plasma histamine level and PGE_2 level in lung tissue during anaphylactic shock: Plasma histamine level and PGE_2 levels in lung tissue were determined in rats either when anaphylactic shock was induced with ovalbumin or when dibutyryl cyclic AMP (Db-c-AMP), indomethacin, indomethacin plus Db-c-AMP, and PGE_2 were given as a pretreatment before the ovalbumin-challenge. Results were summarized in Table 1.

Ovalbumin-challenge produced a significant elevation in the level of plasma histamine ($P<0.001$; Table 1A) and this elevation was inhibited by Db-c-AMP. Indomethacin did

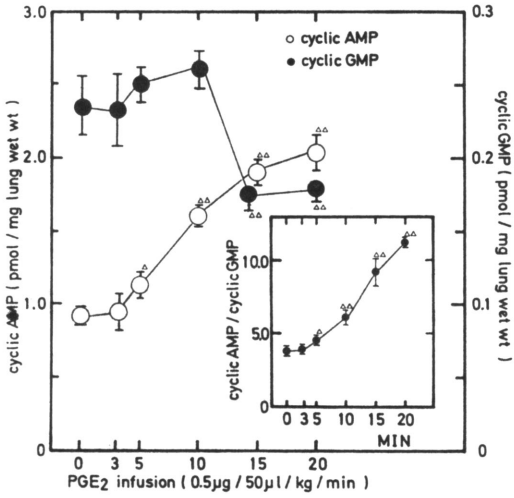

FIG. 2. Influence of PGE_2 infusion on cyclic AMP/cyclic GMP ratio in lung tissue from sensitized rats. Each point represents the mean±S.D. from 5 experiments. Significance of difference from zero-time value assessed by Student's *t*-test $^{\triangle}p<0.01$; $^{\triangle\triangle}p<0.001$.

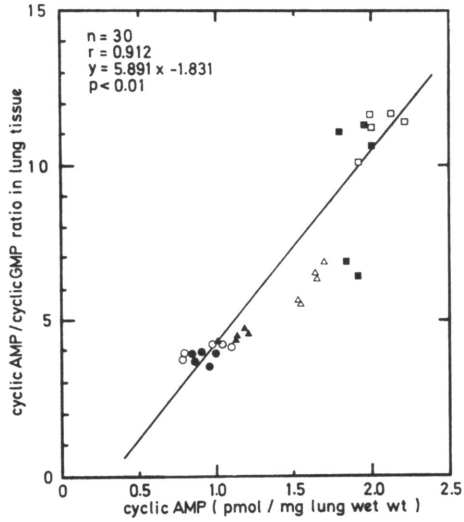

FIG. 3. Relationship between PGE_2 infusion-induced variations in cyclic AMP level and cyclic AMP/cyclic GMP ratio in lung from sensitized rats; the non-treated group (●), PGE_2 infusion for 3 min (○), for 5 min (▲), for 10 min (△), for 15 min (■), for 20 min (□).

TABLE 1. Effects of treatment with dibutyryl cyclic AMP, indomethacin and PGE_2 on ovalbumin-induced histamine changes in plasma or PGE_2 changes in lung from sensitized rats

(A) Histamine

Group and treatment	(n)	Histamine (μg/ml plasma)		
		before ALB	60 sec after ALB	P*
None	(6)	0.094±0.004	0.227±0.018	<0.001
Db-c-AMP 5 mg/kg, i.v.	(6)	0.096±0.006	0.097±0.007	NS
Indomethacin (IDM) 10 mg/kg, i.p.	(6)	0.098±0.007	0.273±0.024	<0.001
IDM+Db-c-AMP	(6)	0.092±0.008	0.235±0.017	<0.001
PGE_2 infusion** 0.5 μg/50 μl/kg/min	(6)	0.093±0.005	0.092±0.006	NS

(B) Prostaglandin E_2

Group and treatment	(n)	PGE_2 (ng/g lung wet wt.)		
		before ALB	60 sec after ALB	P*
None	(6)	14.26±0.64	4.31±0.38	<0.001
Db-c-AMP	(6)	21.84±0.48[a]	20.63±0.37	NS
IDM	(6)	5.19±0.29[a]	3.14±0.37	<0.001
IDM+Db-c-AMP	(6)	6.26±0.33	3.45±0.17	<0.001

ALB=2% ovalbumin 0.2 ml/rat i.v. *P values indicate the significance between values in the treatment before ALB and treatment after ALB. **PGE_2 infusion was continued for 10 min until ALB. Results are the mean±S.E. NS= not significant at level of $p<0.05$. a: $p<0.001$ (VS. line 1)

not influence the ovalbumin-induced elevation of histamine level but did abolish the effect of Db-c-AMP in inhibiting the elevation in plasma histamine. PGE_2 infusion inhibited the elevation in plasma histamine. The level of PGE_2 was decreased significantly by the ovalbumin-challenge ($P<0.001$; Table 1B). Db-c-AMP elevated PGE_2 level significantly ($P<0.001$) compared with that of the non-treated group, and inhibited the decrease of PGE_2 level, as induced by ovalbumin-challenge. Indomethacin decreased the PGE_2 levels significantly ($P<0.001$) compared with the non-treated group and had no influence on the decrease of PGE_2 level as induced by ovalbumin-challenge. The effects of Db-c-AMP were also abolished.

DISCUSSION

Previous studies (4) showed that theophylline significantly improves the survival rate of anaphylactic rats and that this improvement is correlated with elevation of PGE_2 levels in lung tissue. The findings of the present study suggest that an intracellular control mechanism involving cyclic AMP and cyclic GMP during anaphylactic shock depends on the levels of PGE_2, and that PGE_2 regulates the anaphylactic histamine release. It appears that cyclic AMP and cyclic GMP exert opposite effects and this may represent an example of the "Yin-Yang" theory first proposed by Goldberg et al. (13). Haberland (25) previously showed that alteration of cell functions and consequent release of histamine is an important component in the pathogenesis of anaphylactic shock.

In the present study, ovalbumin-challenge elevated plasma histamine levels, and decreased cyclic AMP/cyclic GMP ratio and PGE_2 levels in lung tissue significantly. The histamine level reached a maximum and cyclic AMP/cyclic GMP ratio reached a minimum 60 sec after the challenge, only the PGE_2 level reached a minimum 20 sec earlier (Fig. 1).

With regard to the relationship between PGE_2 and cyclic nucleotides, the following four findings suggest that during anaphylactic shock, there is a decrease of PGE_2 levels in lung tissue which is coincident with a decrease in the cyclic AMP/cyclic GMP ratio: 1) during anaphylactic shock, a decrease in PGE_2 levels precedes a decrease in the cyclic AMP/cyclic GMP ratio (Fig. 1), 2) PGE_2 infusion elevates cyclic AMP levels and cyclic AMP/cyclic GMP ratio in lung tissue in a time-dependent fashion (Fig. 2), 3) Db-c-AMP elevates PGE_2 level in lung tissue significantly (Table 1B), 4) a significant correlation was observed between the elevation of cyclic AMP level and that of cyclic AMP/cyclic GMP ratio in lung tissue both induced by PGE_2 infusion (Fig. 3).

With regard to the relationship between PGE_2 and histamine, anaphylactic elevation of plasma histamine may result from a decrease of PGE_2 level in lung tissue as: 1) the effect of Db-c-AMP which is considered to increase intracellular cyclic AMP levels (8, 26, 27) to which in turn inhibits anaphylactic elevation of plasma histamine, was attributed to the action of Db-c-AMP on increase of PGE_2 level, because the effect was abolished in the concomitant administration with indomethacin (Table 1A, B), a potent inhibitor of PGs biosynthesis (28, 29), 2) anaphylactic elevation of plasma histamine was inhibited when the levels of PGE_2 were maintained at high rates before challenge (Table 1A, B), 3) anaphylactic

elevation of plasma histamine was inhibited by PGE_2 infusion (Table 1A). This is in agreement with findings of other workers in which PGs inhibited the immunologic release of histamine from the lung (30, 31). In addition, we found that anaphylactic shock could be prevented by maintaining PGE_2 levels in lung tissue at a value observed before onset of anaphylactic shock (4).

Therefore, it may be concluded that the onset of anaphylactic shock in rats is due to the elevation of plasma histamine levels which results from a decrease of PGE_2 level in lung tissue rather than to a decrease of cyclic AMP/cyclic GMP ratio, though these decreases are coincident during anaphylactic shock.

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Terfenadine のラットにおける亜急性毒性試験ならびに回復試験

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原　　著

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Abstruct : Subacute Toxicity and Recovery Tests of Terfenadine in Rats

We studied the safety of Terfenadine (TFN), a new type of antihistaminic with no suppressive action on the central nervous system, by performing a subacute toxicity test and recovery test with SD strain rats placed on a 1-month consecutive oral administration regimen of 50mg, 125mg, 250mg and 500mg／kg.

1) TFN had no influence on the behavioral aspect of rats in each administration group.
2) Symptoms such as depilation, epistaxis and diarrhea were noted occasionally in the high dose group.
3) Inhibition of the body weight gain was observed in males and females of the high dose group.
4) An increase in the amount of urine was seen in parallel with the doses of TFN.
5) Findings of hypofunction of the liver was noted in the high dose group.
6) As regards the body weight ratio of organ weights, an increase was observed in the liver of males and females of the high dose group.
7) Administration of TFN exerted no organic influence on main organs.
8) These results indicate that the maximum-non-toxic dose will be somewhere between 125 mg／kg and 250 mg／kg.

抄　　録

中枢神経系抑制作用がない新しいタイプの抗ヒスタミン薬である Terfenadine（ＴＦＮ）について，ＳＤ系ラットにおける 50mg，125 mg，250 mgおよび 500 mg／kg の１ヵ月連続経口投与による亜急性毒性試験ならびに回復試験をおこない，その安全性について検討した。

１）ＴＦＮの各投与群では，ラットの行動学的側面像には影響を与えない。

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2）ＴＦＮの高投与群で脱毛，鼻出血，下痢などの症状が散見された。
3）雌雄のＴＦＮ高投与群で，体重増加の抑制がみられた。
4）ＴＦＮの投与量に平行して尿量の増加が認められた。
5）ＴＦＮの高投与群で肝機能低下の所見が認められた。
6）臓器重量の体重比において，雌雄の高投与群の肝臓に増加がみられた。
7）ＴＦＮ投与による各主要臓器にたいする器質的影響はみとめられない。
8）ＴＦＮ投与による亜急性毒性学的な最大無作用量は，125 mg／kgと 250 mg／kgの中間であろう。

緒　言

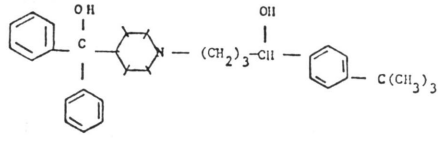
Terfenadine（以下，ＴＦＮと略）はRichardson-Merrell 社によって開発された抗ヒスタミン薬であり，他の抗ヒスタミン薬に比して中枢神経抑制作用をもたないことを特徴とする新しい化合物である[1-3]。その毒性学的検索については，我国においてわずかにラットの急性毒性の報告[4]のみである。そこで，今回，ラットを用いてＴＦＮの１ヵ月間連続経口投与による亜急性毒性試験ならびに回復試験を実施し，その安全性について検討したので報告する。

実験材料および方法

1.　被検薬

Richardson-Merrell 社より提供をうけたＴＦＮは，非水溶性の白色結晶性粉末で，分子量471.66 融点 141～151 °C である。化学的には，α-（4-tert-butylphenyl）-4-（α-hydroxy-α-phenylbenzyl）-1-piperidinebutanol であり，その構造式については Fig. 1 に示した。

Fig. 1　Chemical structure of Terfenadine



$C_{32}H_{41}NO_2$　：　471.66

α-(4-tert-butylphenyl)-4-(α-hydroxy-α-phenylbenzyl)-1-piperidinebutanol

2.　実験動物ならびに飼育条件

実験動物は雌雄の Sprague-Dawley（以下，ＳＤと略）系ラット（Charles River 社）を生後４週令で購入し，温度 23±1 °C，湿度 55±5 %の恒温恒湿の動物室で飼育し，固型飼料（ＣＥ－２，日本クレア）と水道水を自由に摂取させた。なお，１週間の予備飼育後，健康なものをえらび実験に供した。

3.　投与方法，投与期間および投与量

投与量の設定は，急性毒性試験[4]の結果より最大量を 500 mg／kgとし，その最大量に対する公比½で高用量群 250 mg／kg，中間量群 125 mg／kg，なお最少量は50mg／kgとして４群を設定した。ＴＦＮは１匹の投与容量が１mℓ／100 gになるように局方ダイズ油中に懸濁し，投与時ごとに調整した。また，対照群は，局方ダイズ油のみを経口投与した。

薬物の投与は，１日１回，午前10時前後に市販のラット用金属経口ゾンデを用いて30日間連続投与をおこなった。

なお，対照群，250mg／kg投与群および500mg／kg投与群については，１群雌雄各々25例とし，30日間の連続投与終了後，各々雌雄５例を15日間の回復試験に供した。

4.　観察および検査

１）一般症状観察

薬物投与の前後に行動学的側面像と被毛の状態，鼻出血の有無，糞便の性状等について観察した。

２）体重，摂取量の測定

体重および飼料の摂取量は，毎日，薬物投与の直前に測定した。なお，飼料摂取量については毎日一定量を給飼し，その残量よりケージごとに求めた。

３）尿検査

屠殺剖検日の前日から当日にかけて，全例個別に代謝ケージに入れ，新鮮18時間尿を採取し尿量を測定した後，以下の検査をおこなった。

尿蛋白・尿糖・Ketone・bilirubin・urobilinogen・潜血（マルチスティクス，三共），pH（MR試験紙，東洋濾紙），比重（屈折計法，アタゴ光学）。

4） 血液検査

血液学的検査は，屠殺剖検前日に全例尾静脈より採取した血液について実施した。なお，屠殺剖検時に頸動脈より採取した血液を遠心分離し，血清について生化学的検査をおこなった。

（a） 血液学的検査： 赤血球数・白血球数（自動血球計算機，日本光電），hematocrit 値（毛細管遠心法），hemoglobin 量（cyanmethemoglobin 法），白血球百分比（Giemsa 染色塗沫標本）。

（b） 血清生化学的検査： 総 cholesterol（O-phthaladehyde 変法），alkalinephosphatase（phenylphosphate 法），GOT・GPT（Reitman-Frankel 法），総 bilirubin（Evelyn-Malloy 法），尿素窒素（Urease-Indophenol 法），creatinine（Folin-Wu 法），blood sugar（Toluidine ホウ酸法），A／G 比（BCG・ビューレット法），総蛋白（屈折計法，エルマ），Na^+・K^+（炎光分析法，日立508），Cl^-（Schales & Schales 法）。

5） 臓器重量および病理組織学的検索

採血後の動物は主要臓器の肉眼的観察をおこなうとともに，脳（小脳を含む），脳下垂体，胸腺，肺臓，心臓，肝臓，脾臓，腎臓，副腎，睾丸および卵巣をそれぞれ摘出し，各臓器重量の秤量をおこない，さらに相対臓器重量も求めた。

上記の諸臓器に加えて，甲状腺，気管，膵臓，胃，小腸および大腿骨骨髄を摘出した。すべての摘出臓器は10%中性 formaline 液で固定した後，常法に従い，Hematoxylin-Eosin 重染色組織標本を作成し，その組織像について光学顕微鏡下で病理組織学的に検索した。

6） 電子顕微鏡的検索

肝臓，腎臓，肺臓，脾臓，消化管については摘出後，直ちに4 °Cに冷却した2.5%グルタールアルデヒド（リン酸緩衝液 pH 7.3，5.4 %蔗糖を含む），次いで2 %オスミウム酸にて固定した後，エタノール系列にて脱水，Epon-Araldite 包埋した。その組織像について透過型電子顕微鏡（JEM— 100 B，日本電子）にて検索した。

実験結果

A. 亜急性毒性試験

1. 一般症状観察

TFN 50mg／kg，125mg／kg および 250mg／kg 投与群では，雌雄ともにまったく異常が認められなかった。最大用量投与群 500 mg／kg において，雌雄ともに投与後 7 〜10日前後から立毛，被毛光沢消失などの衰弱症状を示すものが数例認められた。しかし，この衰弱症状は漸次回復する傾向を示し，投与20日前後でほとんど正常時の状態に回復した。また，投与 6 日後，雌雄各々 2 例の鼻出血を認め，同時に軟便，下痢が観察された。餌の摂取量では，500 mg／kg 投与群の雌雄において投与開始初期に一過性の低下を示した（Fig. 2, 3, Table 1, 2）。なお，運動抑制は，すべての投与群でみとめられなかった。

2. 体重曲線

TFN最大用量投与群 500 mg／kg の雌雄に体重増加率の軽度の抑制傾向がみられたが，有意の差は認められなかった。それ以外の投与群においては，体重増加抑制はまったく認められなかった（Fig. 4, 5, Table 3, 4）。

3. 死亡例

TFN 500 mg／kg投与群において，投与開始から 8 日目に雌 2 例が，10日目に雄 2 例が死亡した。

4. 尿検査

TFN投与量が増すに従い尿量の増加，比重の減少および尿のアルカリ化傾向が認められた。他の検査項目には変化がみられなかった（Table 5, 6）。

5. 血液検査

（a） 血液学的検査

TFN 500 mg／kg 投与群の雄に好中球の増加傾向が認められたが，他の検査項目においてはまったく変化はみられなかった（Table 7, 8）。

（b） 血清生化学的検査

生化学的検査の結果は， Table 9, 10 に示した。BUNにおいて250 mg／kgおよび500 mg／kg投与群の雌で，対照群に比してわずかな増加が認められた。GOTでは，250 mg／kgおよび500 mg／kg投与群の雄，500 mg／kgの雌でそれぞれ高値を示し，対照群に比して有意の差を認めた。また，GPTにおいても500 mg／kg投与群の雌雄ともに有意な増加が認められた。さらに，雌雄の500 mg／kg投与群でalkalinephosphataseの有意な上昇が認められた。なお，総蛋白，血糖，電解質およびその他の検査所見で若干の変動が認められたが，いずれもTFN投与による影響および投与量と相関する一定の傾向は認められなかった。

6. 臓器重量および病理組織学的検索

（a） 肉眼的所見

いずれの投与群においてもうっ血を伴う肺炎像と肝，腎の軽度のうっ血を認めた以外，特記すべき事項はなかった。

（b） 臓器重量および体重相対重量（体重比）

臓器重量を Table 11, 12 に，体重比を Table 13, 14 に示した。

雌では50mg／kg投与群の左卵巣重量の減少，500 mg／kg投与群の左右卵巣重量の減少および肝重量の増大がみられた。しかし，これらの体重比では，肝のみ有意差が認められたにすぎなかった。雄の場合では，絶対重量になんら差異を認めないが，体重比で250mg／kg投与群の胸腺，500mg／kg投与群の脳，胸腺，肝，脾に増加がみられた。絶対重量および体重比の両者に有意差を認めたのは，雌の500 mg／kg投与群における肝のみであった。

（c） 病理組織学的所見

肺において，対照群，投与群ともにうっ血，無気肺，ほか散発的に肺炎，膿瘍形成，胸膜炎などの炎症変化が認められた。また，肝においても対照群，投与群ともに軽度のうっ血をみる他，少数例に脂肪変性が認められた。腎では軽度のうっ血以外，著変は認められなかった。消化管においては，小腸の被覆粘膜の軽度剥離，小腸および大腸のリンパ装置の一部に Lymphfolliele の hyperplastic な所見がみられたが，対照群とともに生理的な状態の範囲に含まれるものであった。その他の臓器についても検索したが，異常所見は認められなかった（Photo a～h）。

7. 電子顕微鏡的検索

Photo 7, 8 に示したように肝においては，血清生化学的検索でみられた異常と一致する所見は認められず，核周囲に存在する粗面小胞体（nEr）と多数の円形の糸粒体（M）およびその間に豊富なグリコーゲン野（GI）が認められ正常な像であった。また，他の臓器についても Photo 1～6, 9, 10 に示したように異常所見は認められなかった。

B．回復試験

1. 一般症状観察

いずれの投与群においても死亡例はみられなかった。また，投与期間中に観察された症状も回復試験期間中はまったくみられなかった。

2. 体重曲線および摂食量

いずれの投与群においても対照群と同程度の体重増加率を示した。また，餌の摂取量についても対照群とほぼ平行した（Fig. 2～5, Table 15～18）。

3. 尿検査

対照群と投与群との間には，雌雄ともすべての検査項目において有意の差は認められなかった（Table 19）。

4. 血液検査

（a） 血液学的検査

TFN投与終了時の検査において，雄の500mg／kg投与群で好中球の増加傾向がみられたが，休薬期間終了時では変化が認められなかった。他のすべての検査項目においても対照群との間に差異は認められなかった（Table 20, 21）。

（b） 血清生化学的検査

回復試験における検査結果は Table 22, 23 に総括して示した。投与終了時では雌のBUN，雌雄のGOT，GPTおよび alkalinephosphatase に高値が認められたが，休薬期間終了時にお

けるBUNは対照群の値付近まで回復し，有意差は認められなかった。しかし，GOT，GPTおよびalkalinephosphataseの値は，回復傾向がみられるものの完全に回復するまでに至らず高値を示し，対照群との間に有意差が認められた。他の検査項目においては，対照群との間に差異は認められなかった。

5. 臓器重量および病理組織学的検索

（a） 肉眼的所見

肺，肝，腎にみられた軽度のうっ血と，肝にみられた腫大は，休薬期間終了時にまったく観察されなかった。

（b） 臓器重量および体重相対重量（体重比）

回復試験における臓器重量をTable 24, 25に，体重比はTable 26, 27に示した。臓器重量では，対照群および投与群のいずれの臓器についても有意の差異は認められなかった。しかし，体重比において雌雄の500 mg／kg投与群で，肝のみに有意の増加が認められた。

（c） 病理組織学的所見

いずれの投与群においても，対照群と差異が認められず，特記すべき事項はなかった。

考 察

Terfenadine（TFN）をSDラット体重kg当たり1日量50㎎，125㎎，250㎎および500 mgを，それぞれ1ヵ月間経口投与した亜急性毒性試験と，250 mgおよび500 mg／kg投与群の回復試験をおこなった。

その結果，TFNの投与あるいは投与量の増加にともなって観察された所見は，削痩，肝機能低下であった。これ以外にはTFNの毒性と関連すると判断されるものはない。

TFN投与によって観察された一般状態変化の主なものとして，体重増加の抑制または減少，飼料摂取量の減少，削痩，軟便あるいは下痢，脱毛，鼻出血であった。これらの変化が顕著にあらわれたのは，500 mg／kg投与群の雌雄に共通していた。

全投与期間中に死亡したものは500 mg／kg投与群の雌雄2例づつであったが，死亡に先だって著しい体重減少および飼料摂取量の減少を認めた。しかし，剖検による所見からは特に異常を認めなかったため，個体が著しい食欲不振により，衰弱したことに起因するものと考えらる。これらのことからTFN投与が体重減少，飼料摂取の不振，脱毛，鼻出血あるいは下痢の出現頻度，また，出現時期に何らかの形で関与していることは明らかである。しかし，いかなる作用によるものなのか，その機序は不明である。

内臓々器に関しては，体重比において最大用量500 mg／kg投与群の雌雄で増加傾向がみられた。このことは，実重量に大きな変動がみられないことから体重減少による変化と考えられる。しかしながら，500 mg／kg投与群雌雄の体重比において肝の有意な増加と，500 mg／kg投与群雌の肝実重量の有意な増加が認められた。けれども，病理組織学的な所見には何ら器質的変化が認められないので，これら肝の肥大傾向は，TFNが直接的に肝障害作用をもつのか，あるいはTFNの大量投与により全身状態が著しく低下したことによる二次的なものなのかは不明である。2週間の休薬期間終了後（回復試験後）でも，これら体重比における回復がみられなかったことから個体の衰弱のためとは考えにくい。これについては，生化学的検査でもふれる。なお，卵巣の重量減少についての原因は不明である。また，病理組織学的所見において，肺で対照群，投与群ともにうっ血，無気肺，肺炎あるいは膿瘍形成が散発的に認められたが，この変化はTFNの影響によるものと云うより，溶剤としてのダイズ油の誤飲によるものと考えた方が妥当であろう。

血液学的検査においては，投与量の増加にともなって好中球の軽度の増加傾向を示したが，これは肺などに散見された炎症性変化と必ずしも一致しないため，これに関連したものとは考えられない。

臨床生化学的検査において，雌のBUNおよび雌雄のGOT，GPTおよびalkalinephosphataseの量に著明な変動がみられた。雌の250㎎／kgおよび500 mg／kg投与群におけるBUNの上

昇，すなわち，血中尿成分の貯留は腎機能の低下とくに糸球体沪過量の減少が示唆されるが，回復試験終了時に対照群の値の付近まで復したこと，また，病理学的所見で器質的変化が認められなかったことなどから，この上昇は一時的な機能的影響によるものと考えられる。また，GOT，GPTおよび alkalinephosphatase の上昇は，一見肝障害を思わせるものであるが，病理学的所見において特にTFN投与による器質的変化は認められなかった。けれども，肝における 500 mg／kg 投与群雌雄の体重比および雌 500 mg／kg 投与群の実重量がいずれも増加しており，また，回復試験後においても，それらの生化学的所見は高値を示したことなどから投与量が増すと肝機能に何らかの影響を及ぼすものであろう。さらに，尿量はTFNの投与量が増すに従い増加傾向にあり，また，尿のアルカリ化傾向を示した。このことは，HCO_3^- 再吸収の抑制を意味し，尿中への HCO_3^- 排泄にともなって Na^+，K^+ の排泄増加および尿量の増加が予想される。したがって，TFNが尿細管レベルに何らかの作用を及ぼしていることが示唆される。

最後に，これまで述べてきた一般状態，血清生化学的所見，病理組織学的所見等の変動は，回復試験期間中を通じて改善されるものであったが，雌雄の 500 mg／kg 投与群にみられた transamirase および alkalinephosphatase の上昇は，この期間内において正常値への回復がみられず，いぜん肝障害の様相を呈するものであった。また，性差による差異はないものと思われる。以上のような諸点を考慮に入れると，TFNの投与量 250 mg／kg 以内では全身諸臓器，血清生化学的所見および一般状態に著しい変化をおよぼすことがきわめて少ないものと結論される。

結　論

SDラットを用いて，Terfenadine（TFN）50mg，125 ng，250mg および 500 mg／kgの1ヵ月連続経口投与による亜急性毒性試験および回復試験をおこない，以下の結論を得た。

1）　TFNの投与は，行動学的側面像に影響を与えない。

2）　高投与群で，脱毛，鼻出血，下痢等の症状が散見された。

3）　高投与群で雌雄ともに，体重増加の抑制および摂飼量の減少がみられた。

4）　尿量において，投与量に平行した増加が認められた。

5）　血清生化学的検査において，高投与群で肝機能低下の所見が認められた。

6）　臓器重量の体重比において，高投与群の雌雄肝に増加がみられた。

7）　本実験におけるTFNの投与は，各主要臓器にたいして器質的変化を与えない。

8）　TFNの亜急性毒性学的な最大無作用量は 125 mg／kg と 250 mg／kgの中間であろう。

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Fig. 2 Changes in food consumption of male rats receiving TFN for 1 month

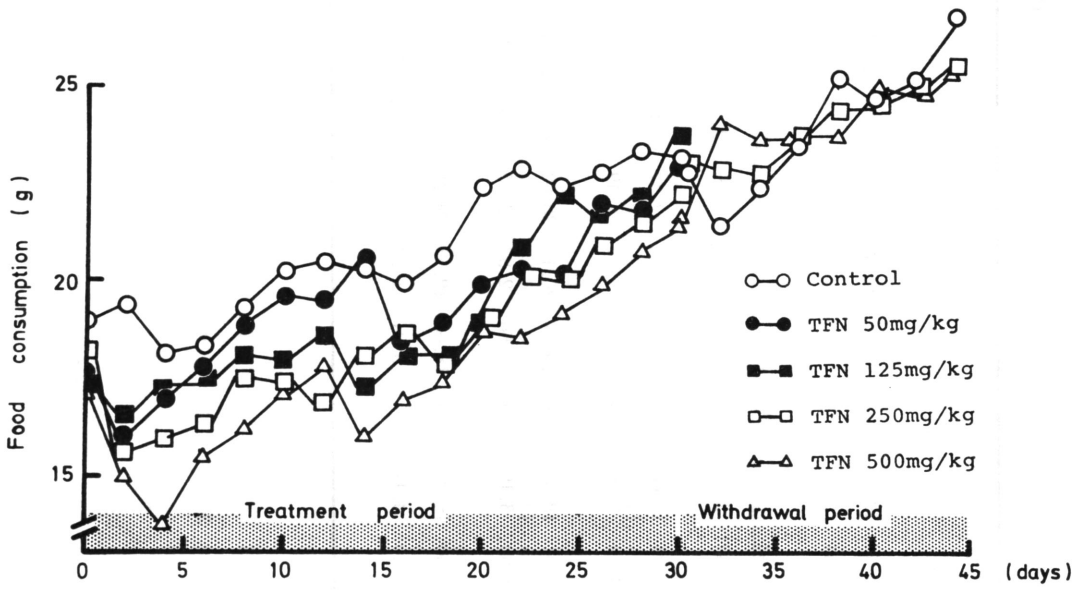

Fig. 3 Changes in food consumption of female rats receiving TFN for 1 month

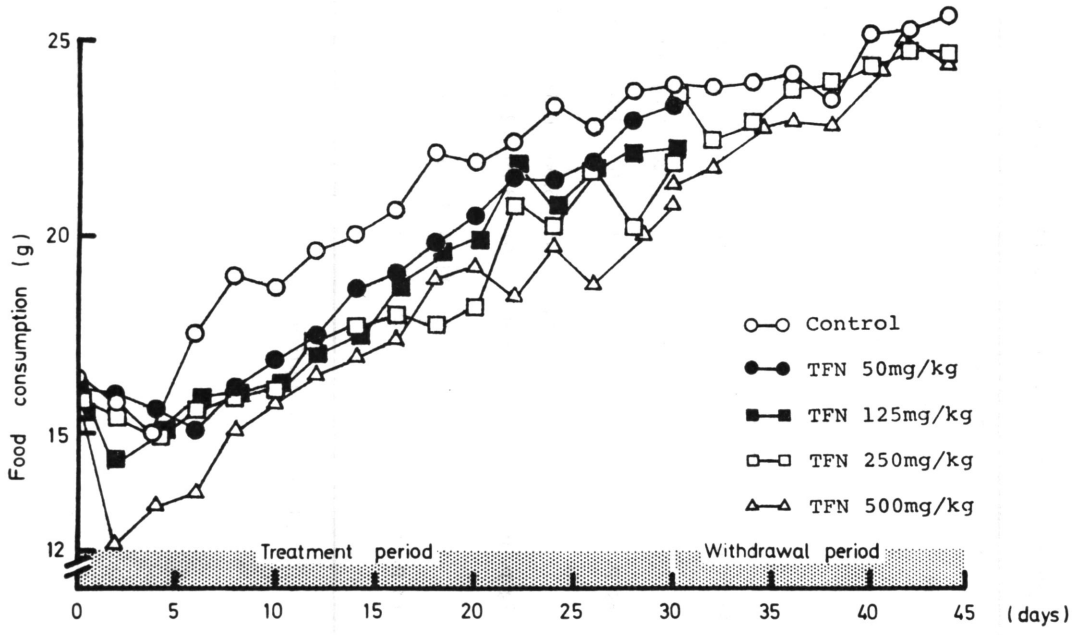

TABLE 1

Food consumption of male rats receiving TFN for 1 month (means ± S. D.)

procedure	No. of rats	Treatment period (days) 0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
control	25)	18.90	19.30	18.13	18.33	19.33	20.26	20.46	20.32	19.94	20.65	22.45	22.89	22.49	22.81	23.44	23.25
	±	1.02	1.15	1.50	2.13	2.38	2.45	1.70	2.54	2.07	3.27	3.50	2.43	3.91	3.44	3.62	3.01
TFN	20)	17.80	16.00	16.95	17.75	18.85	19.58	19.55	20.42	18.48	18.94	19.95	20.23	20.19	22.08	21.94	23.04
50mg／kg p. o.		0.95	1.41	2.17	2.32	2.98	2.46	3.18	3.39	2.13	4.25	4.71	2.26	3.39	2.51	1.65	3.52
TFN	20)	17.52	16.48	17.08	17.65	18.01	17.92	18.54	17.24	18.06	18.00	19.08	20.95	22.37	21.82	22.09	23.82
125mg／kg p. o.		1.12	1.52	2.01	1.83	2.95	3.01	2.55	3.99	2.46	1.98	3.20	3.14	2.49	2.52	2.38	2.46
TFN	25)	18.01	15.65	15.90	16.32	17.48	17.38	16.85	18.01	18.54	17.92	19.01	20.13	20.03	20.92	21.52	22.21
250mg／kg p. o.		1.01	0.94	3.89	2.51	3.21	2.99	2.94	3.88	3.19	3.26	1.66	2.37	2.95	1.92	2.88	2.02
TFN	25)	17.35	14.96	13.77	15.45	16.21	17.07	17.83	16.00	16.95	17.40	18.68	18.53	19.21	19.95	20.84	21.48
500mg／kg p. o.		1.29	1.60	2.86	1.62	2.02	2.10	2.03	4.20	3.44	3.82	3.84	1.22	2.26	3.00	2.17	3.20
							(n=23)	(″)	(″)	(″)	(″)	(″)	(″)	(″)	(″)	(″)	(″)

food consumption : g

TABLE 2

Food consumption of female rats receiving TFN for 1 month (means ± S. D.)

procedure	No. of rats	Treatment period (days) 0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
control	25)	16.33	15.75	15.05	17.55	19.00	18.69	19.68	20.05	20.70	22.20	21.98	22.45	23.40	22.90	23.82	23.95
	±	1.04	2.27	0.84	2.01	1.94	2.33	2.80	2.32	1.53	2.01	1.22	2.93	2.92	0.98	1.43	2.05
TFN	20)	15.69	15.80	15.60	15.10	16.13	16.82	17.52	18.70	19.09	19.88	20.58	21.63	21.51	21.98	22.02	22.49
50mg／kg p. o.		2.34	2.53	1.60	2.11	1.04	1.98	1.91	2.02	1.52	2.28	1.40	2.43	1.52	2.83	2.09	1.90
TFN	20)	16.45	15.49	15.12	15.90	16.01	16.22	17.06	17.49	18.75	19.70	19.90	21.92	20.84	22.05	22.23	22.35
125mg／kg p. o.		1.87	1.21	0.79	2.17	2.14	1.82	1.61	1.97	2.25	2.32	2.80	2.42	0.82	2.15	1.54	2.13
TFN	25)	15.80	14.35	14.95	15.56	15.98	16.05	17.40	17.68	18.01	17.80	18.24	20.83	20.37	21.76	20.25	21.99
250mg／kg p. o.		1.39	1.83	1.58	1.25	1.94	1.72	1.21	1.56	1.55	2.67	2.07	3.24	1.82	2.09	3.64	2.07
TFN	25)	15.60	12.15	13.15	13.49	15.07	15.78	16.50	16.92	17.45	18.95	19.29	18.52	19.82	18.85	20.33	20.87
500mg／kg p. o.		1.22	2.89	1.46	2.58	2.69	2.73	2.31	1.70	1.84	2.16	2.73	2.55	3.38	2.16	2.55	1.51
						(n=23)	(″)	(″)	(″)	(″)	(″)	(″)	(″)	(″)	(″)	(″)	(″)

food consumption : g

Fig. 4 Changes in body weight of male rats receiving TFN for 1 month

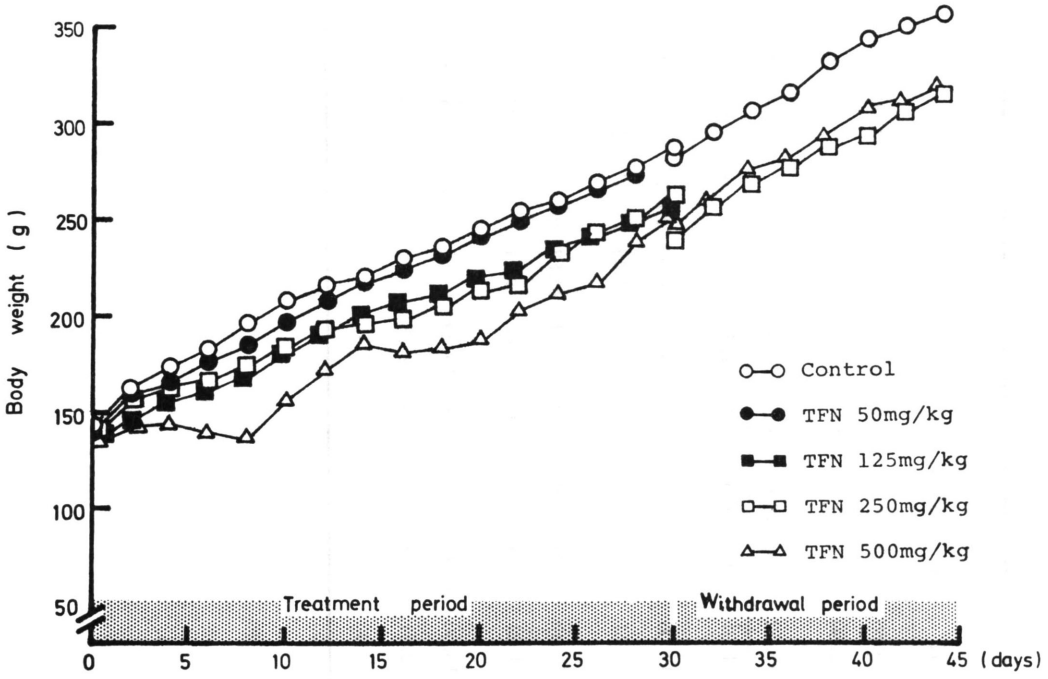

Fig. 5 Changes in body weight of female rats receiving TFN for 1 month

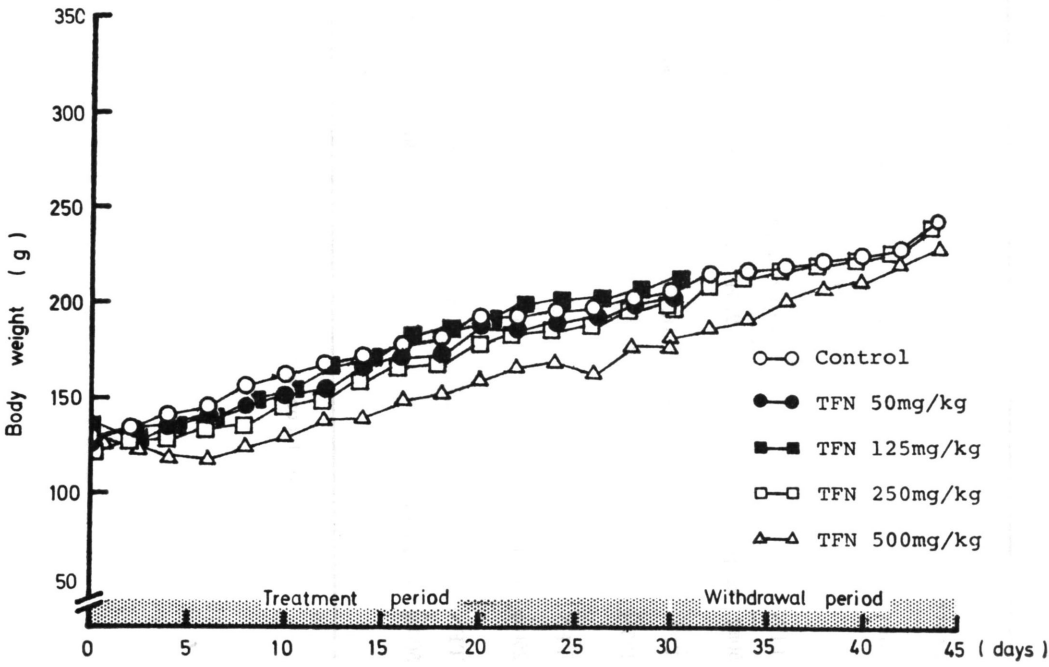

TABLE 3

Changes in body weight of rats receiving TFN for 1 month (means ± S. D.)

Procedure	No. of rats	Days 0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Control	25)	149.15	162.75	173.35	181.75	196.00	208.50	215.95	220.65	230.95	236.40	246.95	256.35	261.80	271.22	279.80	289.95
	±	5.09	5.55	5.62	8.66	10.81	13.58	14.67	15.15	18.54	24.27	25.09	25.21	27.73	29.14	21.52	20.15
TFN	20)	142.70	159.95	165.70	176.10	184.65	195.85	208.90	223.26	227.57	236.11	245.41	249.92	262.91	271.58	281.75	289.14
50mg／kg p. o.		5.62	7.76	8.20	12.19	13.23	13.39	12.82	16.02	19.41	25.02	29.53	31.89	39.38	41.75	31.35	28.05
TFN	20)	136.60	146.95	150.40	160.10	167.65	179.68	189.10	200.47	207.64	212.14	220.0	224.07	234.0	242.40	249.52	258.62
125mg／kg p. o.		9.33	10.68	12.36	13.50	26.77	14.58	20.87	27.35	29.75	36.40	39.52	32.40	28.21	29.93	26.04	27.14
TFN	25)	145.00	156.45	159.35	166.45	175.42	184.31	190.52	198.31	199.47	204.76	215.37	218.68	235.07	245.85	251.84	264.52
250mg／kg p. o.		7.68	10.53	15.84	19.74	20.39	19.76	24.92	29.01	28.07	31.04	29.21	27.48	28.28	25.61	29.45	26.85
TFN	25)	135.45	142.80	143.75	139.50	137.80	156.33	173.16	186.33	181.61	184.05	188.06	204.00	213.70	219.75	240.87	251.09
500mg／kg p. o.		5.03	8.12	9.73	17.09	20.98	17.65	18.24	17.19	22.17	25.53	29.92	30.12	25.01	22.54	18.86	18.12
							(n=23)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)

body weight : g

TABLE 4

Changes in body weight of rats receiving TFN for 1 month (means ± S. D.)

procedure	No. of rats	Days 0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
Control	25)	129.25	132.50	140.45	145.50	156.00	162.50	168.00	172.50	178.72	180.33	193.53	193.65	197.94	198.47	204.52	207.91
	±	8.43	8.06	8.53	11.72	13.97	10.68	9.68	9.97	12.63	13.39	10.09	12.49	10.32	11.40	12.11	10.04
TFN	20)	124.95	130.25	134.40	141.35	146.50	152.84	153.95	164.53	171.11	172.90	189.40	192.80	193.06	197.36	203.31	207.41
50mg／kg p. o.		9.51	9.97	13.00	13.04	15.22	15.06	18.08	16.33	18.71	21.45	15.77	17.91	23.72	25.99	19.37	16.42
TFN	20)	127.45	132.95	137.21	147.00	152.16	157.21	164.00	169.32	179.00	180.22	193.06	198.78	200.88	203.18	205.88	209.65
125mg／kg p. o.		9.11	17.60	12.75	15.71	21.12	22.11	24.29	25.83	24.33	25.39	25.59	27.57	19.90	28.29	16.60	12.30
TFN	25)	126.50	127.15	128.55	133.50	137.95	144.45	151.05	158.79	167.00	168.41	179.14	186.93	190.57	198.64	204.67	208.42
250mg／kg p. o.		7.57	8.57	8.93	11.11	12.04	13.34	13.31	10.49	11.19	13.91	11.25	10.54	10.33	9.34	16.68	12.01
TFN	25)	125.40	125.05	119.20	117.45	123.72	129.28	137.18	139.82	148.06	151.82	159.12	165.88	169.53	163.29	176.67	178.54
500mg／kg p. o.		5.17	6.63	8.48	11.62	11.47	15.71	18.93	21.26	23.91	26.22	20.39	22.97	22.34	26.76	26.98	19.87
						(n=23)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)	(〃)

body weight : g

TABLE 5

Examination of the urine (No. 1)

procedure	No. of rats	administrated rats			No. of rats	recovered rats		
		urine volume (ml／18hr.)	urinary pH	urine density		urine volume (ml／18hr.)	urinary pH	urine density
MALE								
control	20	6.6±0.5	6.5±0.1	1.066±0.004	5	10.2±2.7	7.0±0.3	1.050±0.013
50mg／kg p. o.	20	8.9±0.8	7.1±0.2	1.052±0.005				
125mg／kg p. o.	20	7.9±1.2	6.5±0.2	1.055±0.008				
250mg／kg p. o.	20	9.4±1.7	7.0±0.3	1.048±0.005	5	9.5±2.6	6.5±0.3	1.052±0.012
500mg／kg p. o.	18	9.8±0.5	7.1±0.2	1.056±0.005	5	6.2±1.4	6.7±0.3	1.052±0.012
FEMALE								
control	20	6.8±0.7	6.7±0.1	1.063±0.005	5	9.1±1.6	7.2±0.2	1.058±0.005
50mg／kg p. o.	20	7.9±0.5	7.1±0.2	1.064±0.004				
125mg／kg p. o.	20	8.3±0.9	6.9±0.2	1.049±0.005				
250mg／kg p. o.	20	9.6±0.9	7.1±0.1	1.047±0.005	5	5.4±0.6	7.0±0.1	1.084±0.006
500mg／kg p. o.	18	9.6±1.2	7.2±0.2	1.051±0.007	5	6.5±0.5	7.3±0.3	1.073±0.001

TABLE 6

Examination of urine (No. 2)

procedure	No. of rats	protein			glucose		ketone		bilirubin		benzidine test		urobilinogen	
		(±)	(+)	(++)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(±)	(+)
MALE														
control	20	2	11	7	20	0	20	0	20	0	20	0	20	0
50mg／kg p. o.	20	2	14	4	20	0	20	0	20	0	20	0	20	0
150mg／kg p. o.	20	4	14	2	20	0	20	0	20	0	20	0	20	0
250mg／kg p. o.	20	4	14	2	20	0	20	0	20	0	20	0	20	0
500mg／kg p. o.	18	2	14	2	18	0	18	0	18	0	18	0	18	0
FEMALE														
control	20	4	15	1	20	0	20	0	20	0	20	0	20	0
50mg／kg p. o.	20	5	13	2	20	0	20	0	20	0	20	0	20	0
125mg／kg p. o.	20	7	13	0	20	0	20	0	20	0	20	0	20	0
250mg／kg p. o.	20	8	12	0	20	0	20	0	20	0	20	0	20	0
500mg／kg p. o.	18	4	13	1	18	0	18	0	18	0	18	0	18	0

TABLE 7

Hematological findings in male rats treated orally with TFN for 1 month

	Groups (Mean ± S. E.)				
	control	59mg／kg／day p. o	125mg／kg／day p. o	250mg／kg／day p. o	500mg／kg／day p. o
hemoglobin (g／dl)	15.0± 0.2	16.3± 0.3	14.9± 0.4	15.4± 0.2	14.4± 0.4
hematocrit (%)	45.2± 0.3	43.4± 0.8	42.9± 0.9	43.1± 0.7	45.4± 0.6
erythrocytes ($\times 10^4$／mm^3)	613 ±26	643 ±15	659 ±23	614 ±13	614 ±17
leucocytes ($\times 10^2$／mm^3)	128 ± 4	125 ± 6	117 ± 7	123 ± 6	123 ± 3
bosophils (%)	0	0	0	0	0.2± 0.1
eosinophils (%)	0.4± 0.2	0.6± 0.2	0.6± 0.2	0.4± 0.2	0.2± 0.1
lymphocytes (%)	84.1± 1.4	84.6± 3.2	83.3± 4.7	81.6± 2.2	80.8± 2.8
monocytes (%)	1.8± 0.4	0.9± 0.3	1.0± 0.2	2.2± 0.3	1.7± 0.4
neutrophils (%)	13.7± 0.7	13.9± 0.5	15.1± 0.7	15.8± 0.6	17.1± 0.6

TABLE 8

Hematological findings in female rats treated orally with TFN for 1 month

	Groups (Mean ± S. E.)				
	control	50mg／kg／day p. o	125mg／kg／day p. o	250mg／kg／day p. o	500mg／kg／day p. o
hemoglobin (g／dl)	14.6± 0.2	14.8± 0.2	13.0± 0.2	13.5± 0.3	14.5± 0.3
hematocrit (%)	40.6± 0.7	38.6± 0.6	38.8± 1.0	39.1± 0.9	42.0± 0.9
erythrocytes ($\times 10^4$／mm^3)	650 ±10	640 ±15	663 ±11	679 ±12	683 ±25
leucocytes ($\times 10^3$／mm^3)	135 ±34	111 ±41	113 ±18	136 ±36	110 ±28
basophyls (%)	0	0.3± 0.1	0	0	0.1± 0.1
eosinophils (%)	0.2± 0.1	0.8± 0.1	0.5± 0.1	0.2± 0.1	0.3± 0.1
lymphocytes (%)	87.5± 1.3	83.5± 1.1	86.6± 1.7	83.5± 1.7	86.4± 1.9
monocytes (%)	1.0± 0.3	1.9± 0.2	0.8± 0.8	1.1± 0.3	0.3± 0.2
neutrophils (%)	11.3± 0.5	13.5± 0.5	12.1± 0.6	15.2± 0.6	12.9± 0.7

TABLE 9

Blood biochemical findings in rats treated orally with TFN for 1 month (No. 1)

procedure	No. of rats	Total Protein (g/dl)	A/G	Glucose (mg/dl)	BUN (mg/dl)	Total Cholesterol (mg/dl)	GOT (K-M unit)	GPT (K-M unit)
MALE								
control	20	6.00±0.08	1.22±0.08	162.2± 5.2	20.9±1.0	70.8±1.8	59.7±3.0	27.8±2.8
50mg/kg p. o.	20	5.80±0.09	1.33±0.07	166.3± 8.1	20.8±0.9	69.2±1.9	61.9±6.0	26.4±2.1
125mg/kg p. o.	20	5.99±0.06	1.20±0.07	163.7± 8.7	19.6±1.3	69.1±2.2	63.4±3.4	28.0±3.3
250mg/kg p. o.	20	5.92±0.05	1.21±0.07	151.0±12.2	19.2±1.0	70.9±2.5	76.3±3.5*	30.4±3.1
500mg/kg p. o.	18	5.72±0.07	1.32±0.10	166.8± 5.6	21.1±0.9	68.2±2.4	84.2±5.6**	41.0±7.0*
FEMALE								
control	20	5.78±0.05	1.22±0.04	199.9±10.7	15.9±0.5	67.5±1.7	62.3±3.0	21.3±1.3
50mg/kg p. o.	20	5.88±0.06	1.14±0.05	198.8± 7.8	16.6±0.4	67.6±2.6	69.8±4.0	22.6±1.8
125mg/kg p. o.	20	5.66±0.04	1.27±0.06	194.1± 9.0	15.8±0.5	69.8±4.2	67.4±3.8	25.6±2.3
250mg/kg p. o.	20	5.81±0.06	1.25±0.05	202.1± 7.6	19.4±1.0*	68.7±3.4	69.3±7.4	25.0±5.9
500mg/kg p. o.	18	5.93±0.03	1.25±0.03	206.0± 6.2	20.2±0.7**	67.2±4.6	87.1±7.1**	33.3±3.6**

Mean ± S. E.

*: Significant difference from control ($p<0.05$)

**: Significant difference from control ($p<0.01$)

TABLE 10

Blood biochemical findings in rats treated orally with TFN for 1 month (No. 2)

procedure	No. of rats	ALP (K-A unit)	Creatinine (mg/dl)	Total Bilirubin (mg/dl)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)
MALE							
control	20	30.1±2.2	0.69±0.05	0.21±0.10	127.4±1.3	3.56±0.09	108.7±1.6
50mg/kg p. o.	20	29.9±2.5	0.64±0.04	0.24±0.05	128.4±2.5	3.74±0.11	108.3±1.1
125mg/kg p. o.	20	30.3±3.0	0.58±0.05	0.23±0.04	131.6±1.8	3.73±0.12	113.6±1.1
250mg/kg p. o.	20	32.0±2.2	0.60±0.07	0.29±0.07	131.2±2.6	3.70±0.09	108.3±1.5
500mg/kg p. o.	18	40.9±2.3 *	0.59±0.04	0.25±0.03	134.9±1.5	3.72±0.14	109.2±1.3
FEMALE							
control	20	29.9±2.1	0.63±0.09	0.25±0.11	131.8±1.0	3.62±0.07	109.6±0.6
50mg/kg p. o.	20	31.6±2.3	0.60±0.02	0.23±0.07	129.3±2.3	3.79±0.09	107.9±0.7
125mg/kg p. o.	20	31.2±2.0	0.66±0.03	0.24±0.04	132.1±2.9	3.88±0.19	109.9±0.6
250mg/kg p. o.	20	32.4±2.9	0.67±0.01	0.27±0.05	134.6±1.3	3.78±0.15	109.0±1.4
500mg/kg p. o.	18	41.6±2.1 *	0.64±0.05	0.29±0.04	127.0±2.4	3.66±0.14	108.4±1.3

Mean ± S. E.

*: Significant difference from control ($p<0.05$)

TABLE 11

Organ weight in male rats treated orally with TFN for 1 month

	Control	50mg／kg	125mg／kg	250mg／kg	500mg／kg
No. of rats	20	20	20	20	18
body weight (g)	290.0 ±20.15	289.1 ±28.05	258.6 ±27.14	264.5 ±26.85	251.1 ±18.12
brain (g)	1.90± 0.079	1.87± 0.082	1.85± 0.062	1.86± 0.082	1.88± 0.066
hypophysis (mg)	8.9 ± 2.40	8.9 ± 1.53	8.7 ± 0.74	8.3 ± 0.99	8.6 ± 1.14
thymus (g)	0.54± 0.134	0.61± 0.367	0.51± 0.085	0.65± 0.230	0.67± 0.187
lung (g)	1.66± 0.144	1.71± 0.256	1.64± 0.132	1.62± 0.161	1.50± 0.190
heart (g)	0.98± 0.095	1.03± 0.174	0.94± 0.096	0.98± 0.124	0.94± 0.100
liver (g)	9.19± 1.400	10.25± 1.841	9.23± 1.464	9.09± 1.636	10.46± 1.463
spleen (g)	0.73± 0.113	0.82± 0.195	0.74± 0.158	0.75± 0.149	0.89± 0.224
kidney (left) (g)	1.10± 0.105	1.13± 0.145	1.06± 0.128	1.06± 0.064	1.14± 0.132
(right) (g)	1.13± 0.112	1.14± 0.134	1.07± 0.106	1.08± 0.065	1.14± 0.132
adrenal (left) (mg)	26.3 ± 2.60	26.2 ± 3.26	24.7 ± 4.69	25.8 ± 4.64	25.4 ± 2.84
(right) (mg)	25.1 ± 3.67	26.4 ± 3.36	22.4 ± 5.27	26.5 ± 4.48	25.5 ± 3.24
testis (left) (g)	1.47± 0.092	1.46± 0.130	1.46± 0.208	1.46± 0.105	1.43± 0.113
(right) (g)	1.47± 0.083	1.48± 0.156	1.46± 0.200	1.48± 0.119	1.42± 0.113

Results are given means ± S. D.

TABLE 12

Organ weight in female rats treated orally with TFN for 1 month

	Control	50mg／kg	125mg／kg	250mg／kg	500mg／kg
No. of rats	20	20	20	20	18
body weight (g)	207.9 ±10.04	207.4 ±16.42	209.7 ±12.30	208.4 ±12.01	178.5 ±19.87
brain (g)	1.81± 0.051	1.79± 0.085	1.75± 0.045	1.76± 0.081	1.75± 0.085
hypophysis (mg)	8.4 ± 1.27	8.4 ± 2.09	8.2 ± 1.45	8.0 ± 1.26	8.1 ± 1.61
thymus (g)	0.67± 0.181	0.64± 0.440	0.61± 0.102	0.63± 0.124	0.62± 0.167
lung (g)	1.56± 0.233	1.60± 0.332	1.66± 0.220	1.52± 0.253	1.40± 0.202
heart (g)	0.78± 0.070	0.80± 0.098	0.80± 0.084	0.81± 0.079	0.78± 0.110
liver (g)	7.85± 1.092	7.69± 1.143	7.65± 0.791	8.75± 0.726	9.15± 1.329
spleen (g)	0.62± 0.145	0.67± 0.172	0.62± 0.119	0.63± 0.081	0.66± 0.152
kidney (left) (g)	0.83± 0.099	0.83± 0.112	0.86± 0.095	0.79± 0.089	0.78± 0.152
(right) (g)	0.85± 0.099	0.84± 0.086	0.87± 0.094	0.80± 0.091	0.80± 0.108
adrenal (left) (mg)	26.0 ± 5.74	27.6 ± 4.14	26.6 ± 7.17	26.8 ± 4.92	28.4 ± 5.40
(right) (mg)	25.0 ± 7.01	25.9 ± 4.68	26.8 ± 6.40	26.4 ± 5.65	29.8 ± 5.31
ovary (left) (mg)	95.7 ±18.09	73.8 ±18.02*	76.1 ±28.24	74.4 ±20.61	72.4 ±18.38*
(right) (mg)	93.4 ±15.67	76.1 ±17.29	72.9 ±26.57	79.1 ±23.58	72.2 ±15.99*

Results are given means ± S. D.

*: Significant difference from control ($p<0.05$)

TABLE 13

Relative organ weight in male rats treated orally with TFN for 1 month

	Control	50mg／kg	125mg／kg	250mg／kg	500mg／kg
No. of rats	20	20	20	20	18
body weight (g)	290.0 ±20.15	289.1 ±28.05	258.6 ±27.14	264.5 ±26.85	251.1 ±18.12
brain	0.64± 0.050	0.64± 0.078	0.73± 0.083	0.70± 0.116	0.76± 0.058*
hypophysis **	3.08± 0.562	3.09± 0.362	3.35± 0.524	3.16± 0.250	3.42± 0.669
thymus	0.19± 0.061	0.21± 0.144	0.20± 0.079	0.25± 0.117*	0.28± 0.104*
lung	0.57± 0.064	0.59± 0.089	0.63± 0.148	0.62± 0.068	0.60± 0.079
heart	0.34± 0.026	0.36± 0.063	0.37± 0.045	0.39± 0.085	0.37± 0.064
liver	3.17± 0.329	3.55± 0.429	3.58± 0.452	3.46± 0.462	4.19± 0.440*
spleen	0.25± 0.043	0.28± 0.077	0.29± 0.068	0.29± 0.049	0.36± 0.132*
kidneys (left)	0.38± 0.027	0.39± 0.028	0.42± 0.039	0.41± 0.029	0.45± 0.072
(right)	0.39± 0.025	0.39± 0.021	0.41± 0.042	0.41± 0.043	0.45± 0.065
adrenal (left) **	9.16± 1.087	9.16± 2.361	9.55± 2.281	9.95± 3.733	10.12± 1.022
(right) **	8.69± 1.330	9.13± 2.049	8.96± 2.413	10.02± 3.304	10.16± 1.370
testis (left)	0.51± 0.054	0.51± 0.059	0.56± 0.103	0.55± 0.049	0.57± 0.051
(right)	0.51± 0.049	0.51± 0.063	0.56± 0.111	0.56± 0.056	0.57± 0.047

Results are given means ± S. D.

Relative organ weight = g／100g body weight　** = mg／100g body weight

*: Significant difference from control ($p<0.05$)

TABLE 14

Relative organ weight in female rats treated orally with TFN for 1 month

	Control	50mg／kg	125mg／kg	250mg／kg	500mg／kg
No. of rats	20	20	20	20	18
body weight (g)	207.9 ±10.04	207.4 ±16.42	209.7 ±12.30	208.4±12.01	231.2 ± 8.64
brain	0.87± 0.087	0.86± 0.091	0.84± 0.083	0.85± 0.099	0.80± 0.059
hypophysis **	4.05± 0.880	4.05± 0.709	3.92± 0.629	3.86± 0.976	3.60± 0.87
thymus	0.32± 0.085	0.31± 0.084	0.30± 0.044	0.30± 0.072	0.26± 0.052
lung	0.76± 0.073	0.77± 0.140	0.79± 0.094	0.74± 0.104	0.71± 0.092
heart	0.38± 0.029	0.39± 0.036	0.38± 0.031	0.39± 0.050	0.36± 0.006
liver	3.79± 0.409	3.72± 0.269	3.69± 0.256	4.30± 0.372	3.71± 0.141*
spleen	0.30± 0.062	0.32± 0.086	0.30± 0.037	0.30± 0.057	0.25± 0.026
kidney (left)	0.40± 0.036	0.40± 0.037	0.41± 0.024	0.38± 0.039	0.35± 0.009
(right)	0.41± 0.036	0.41± 0.033	0.41± 0.031	0.38± 0.041	0.35± 0.0012
adrenal (left) **	12.53± 2.768	13.32± 2.913	13.08± 3.419	12.95± 3.501	11.85± 1.901
(right) **	12.17± 3.066	12.52± 2.967	13.18± 3.971	12.86± 3.217	11.43± 1.635
ovary (left) **	46.18± 9.732	35.59± 9.388	37.28±10.296	35.70± 7.984	32.30± 5.020
(right) **	44.94± 8.504	36.71± 6.901	35.76±12.592	37.98± 8.452	32.45± 2.747

Results are given means ± S. D.

Relative organ weight = g／100g body weight ** = mg／100g body weight

*: Significant difference from control ($p<0.05$)

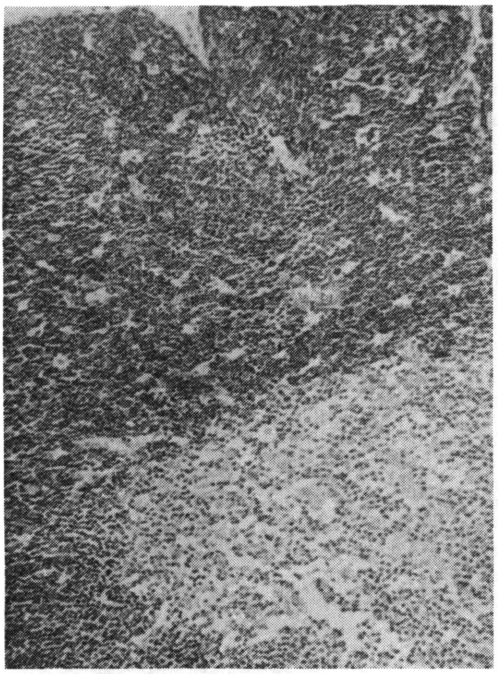

Photo a) The thymus of male rat (control) : No abnormal findings (x 100)

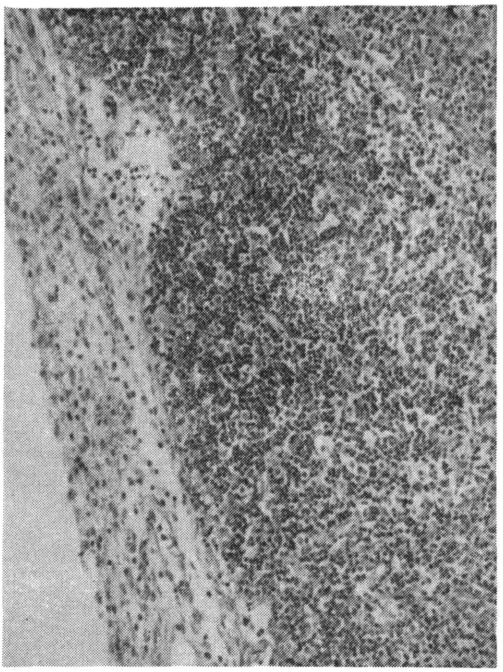

Photo b) The thymus of male rat (TFN 50mg／kg p. o.) : No abnormal findings (x 100)

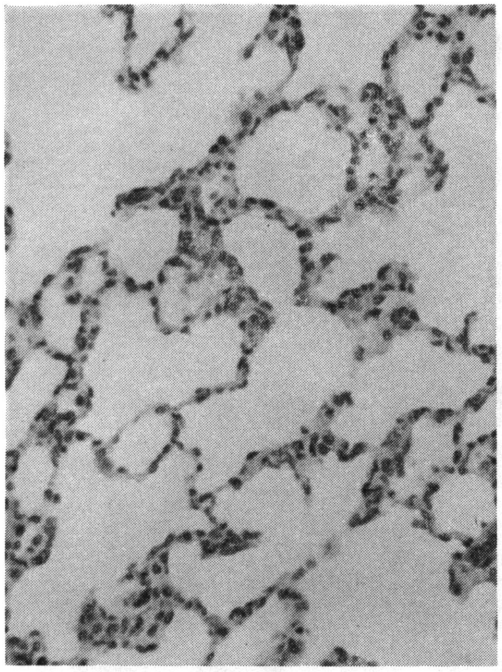

Photo c) The lung of male rat (control) : No abnormal findings (x 200)

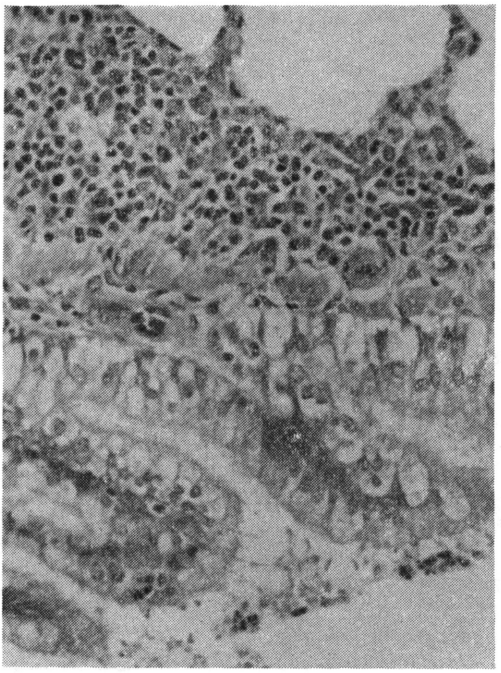

Photo d) The lung of male rat (TFN 500mg ／kg p. o.) : No abnormal findings (x 200)

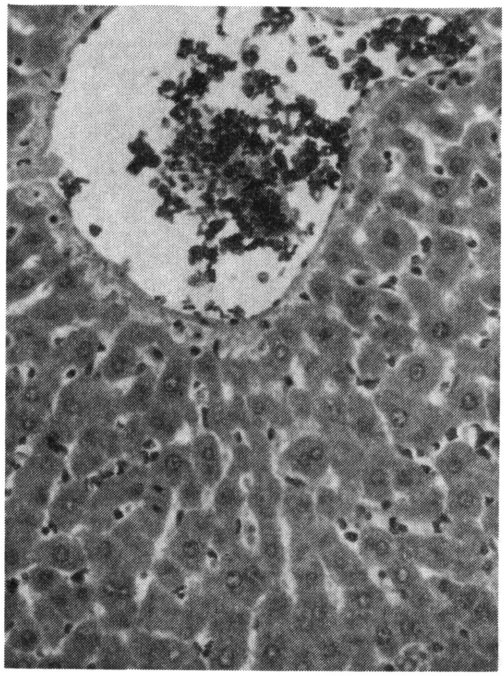

Photo e) The liver of male rat (control) : No abnormal findings (x 200)

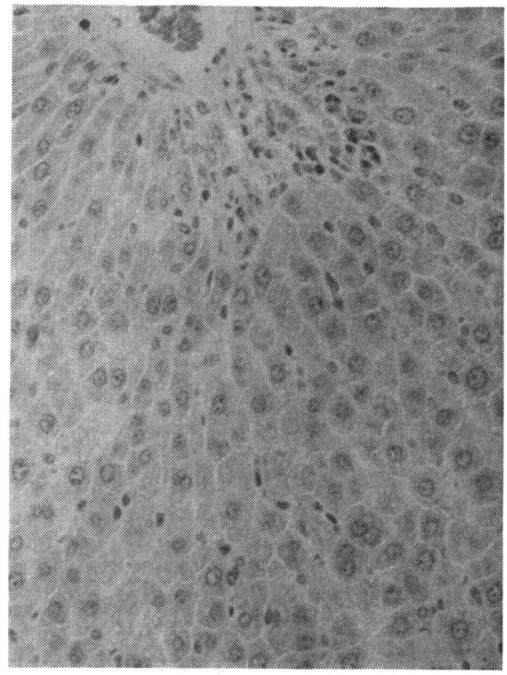

Photo f) The liver of male rat (TFN 250mg /kg p. o.) : No abnormal findings (x 200)

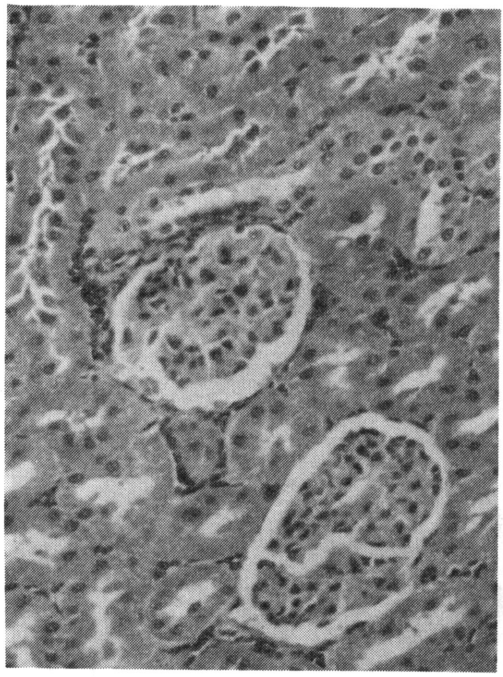

Photo g) The kidney of male rat (control) : No abnormal findings (x 200)

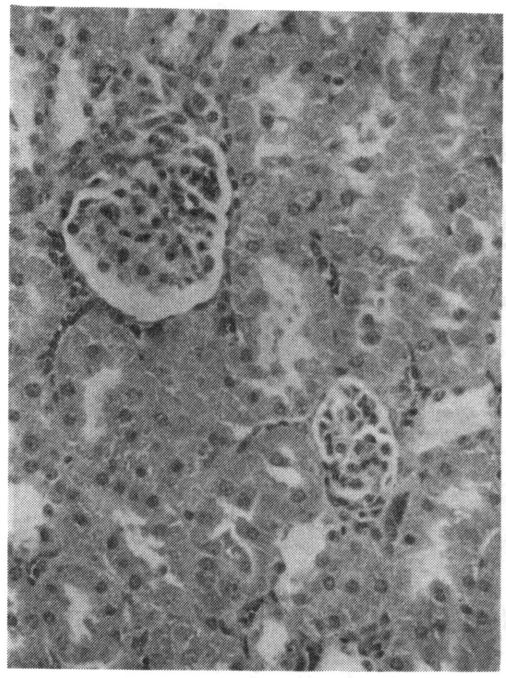

Photo h) The kidney of male rat (TFN 500 mg/kg p. o.) : No abnormal findings (x200)

Photo 1

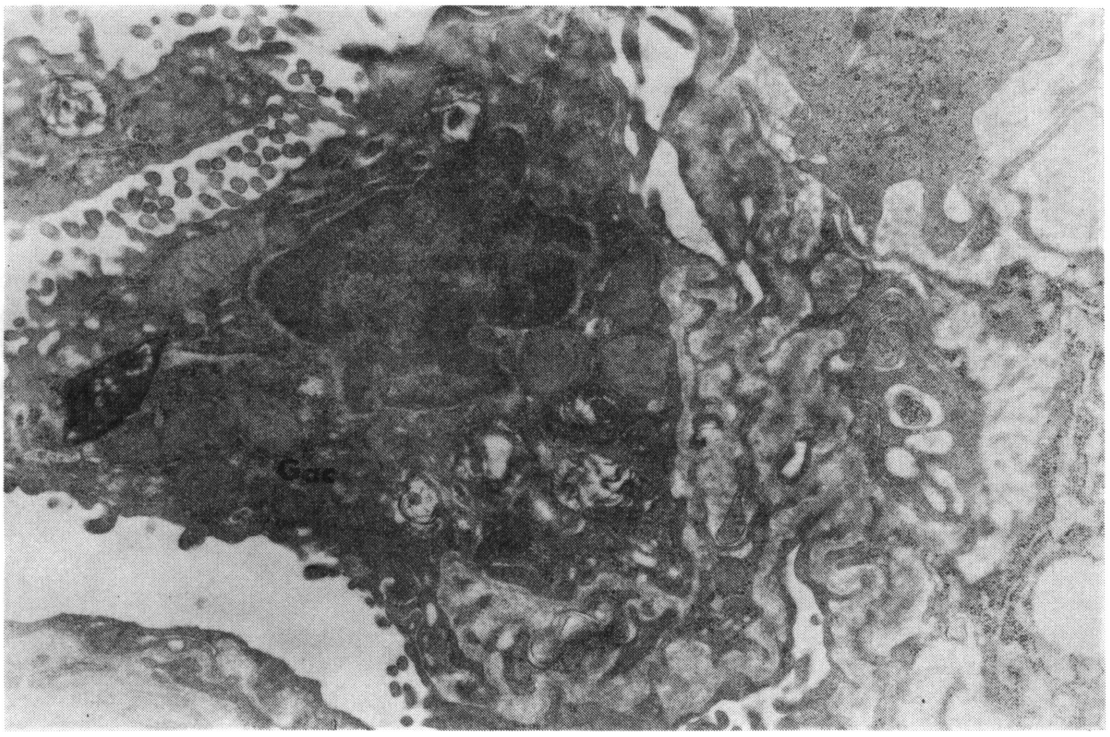

Photo 2

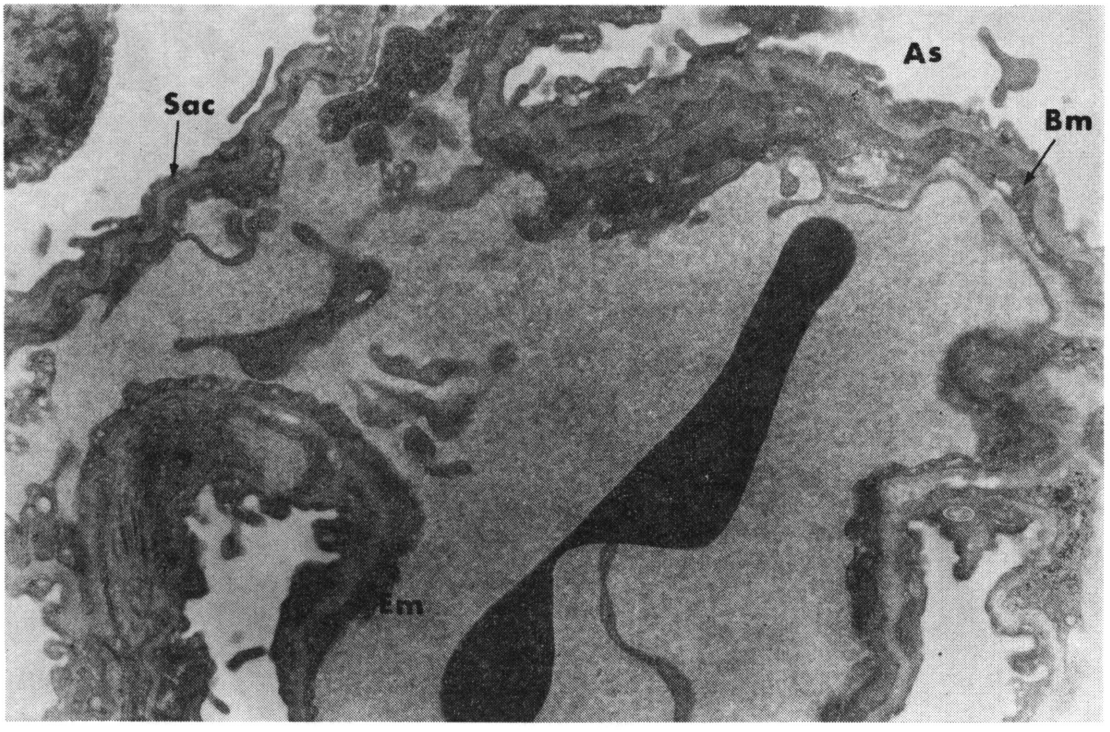

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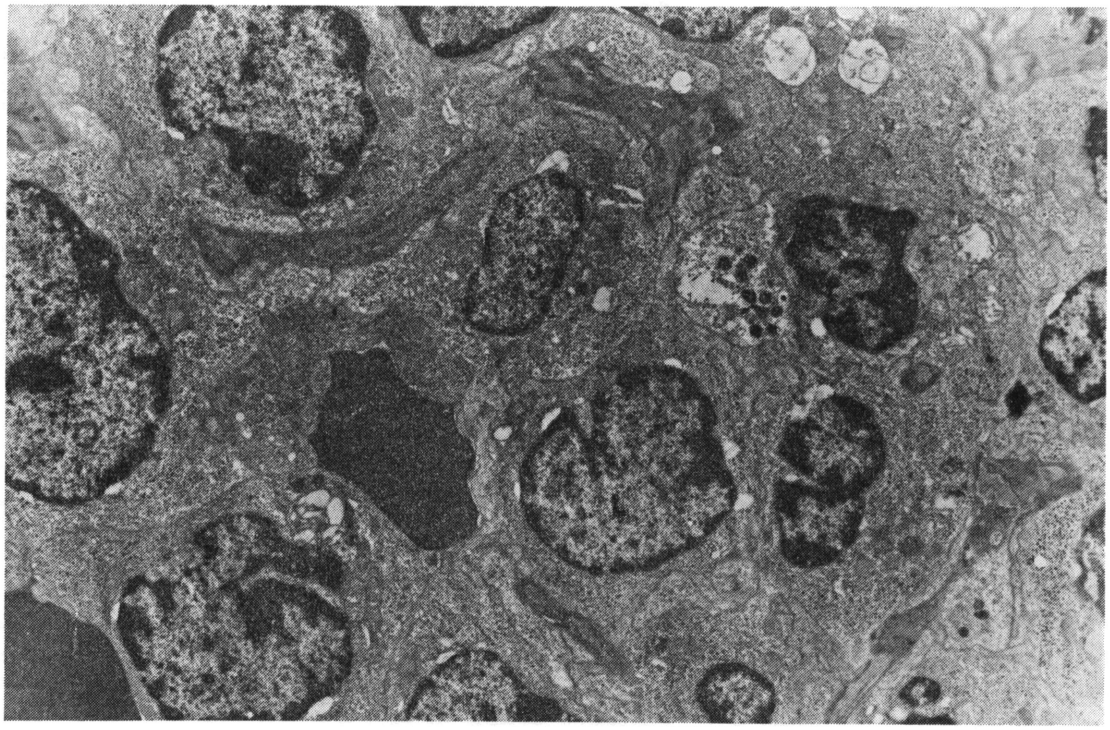
Photo 3



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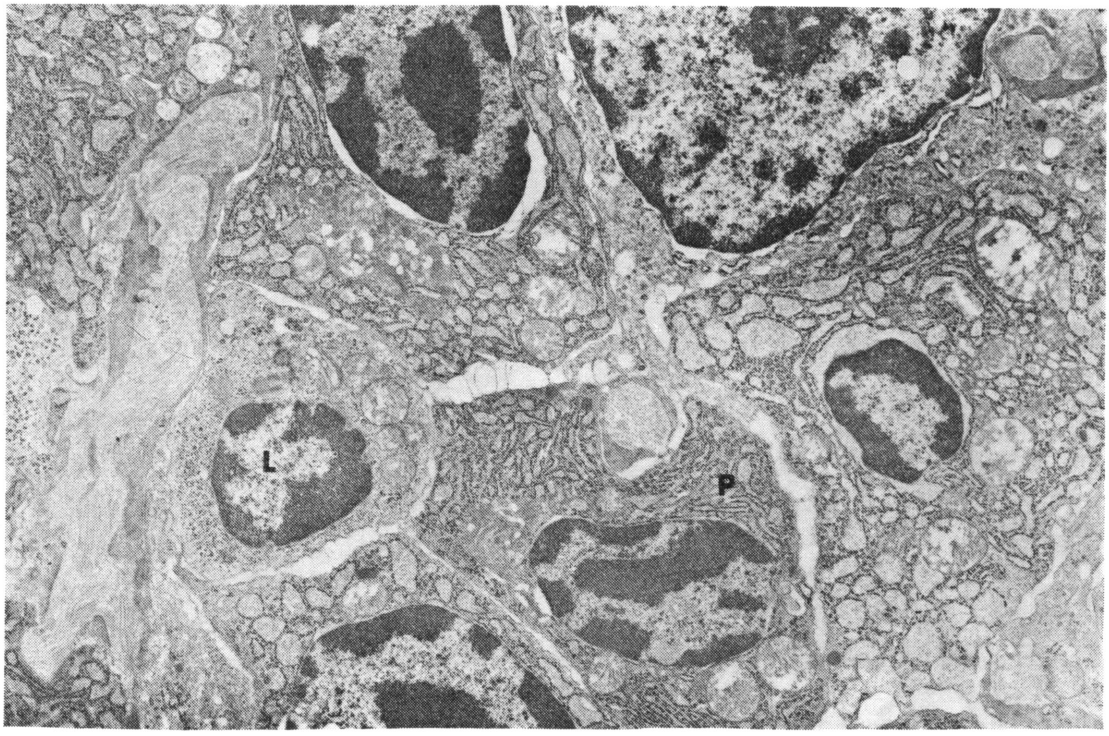

Photo 5

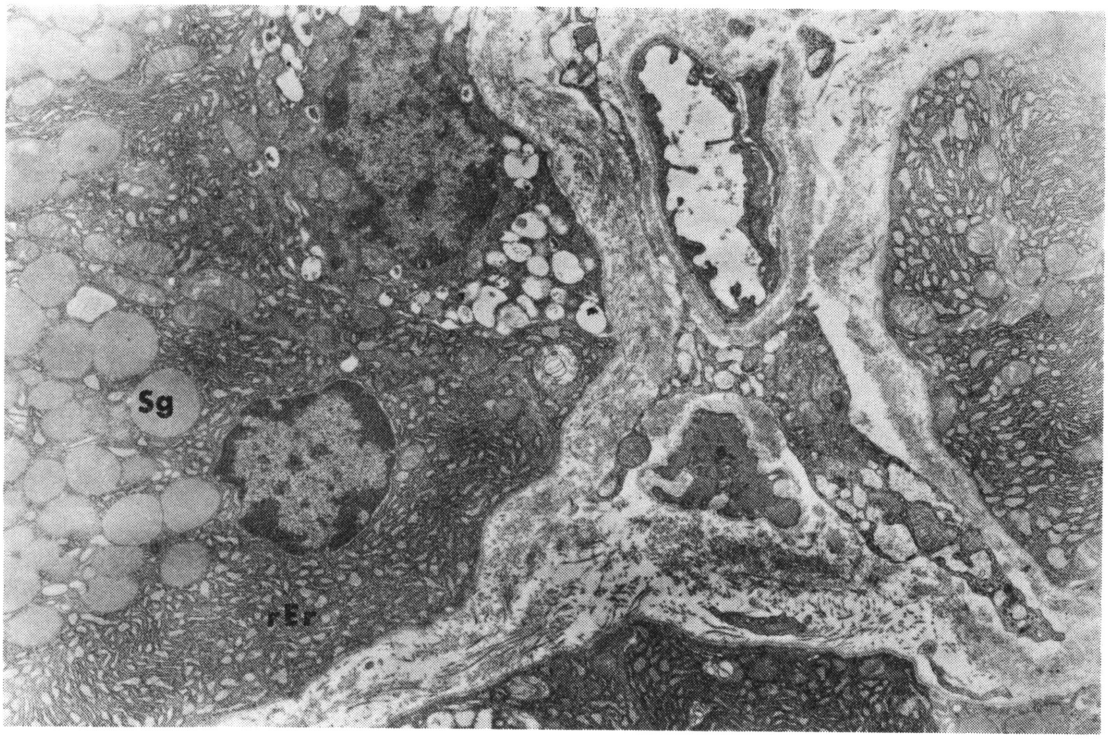

Photo 6

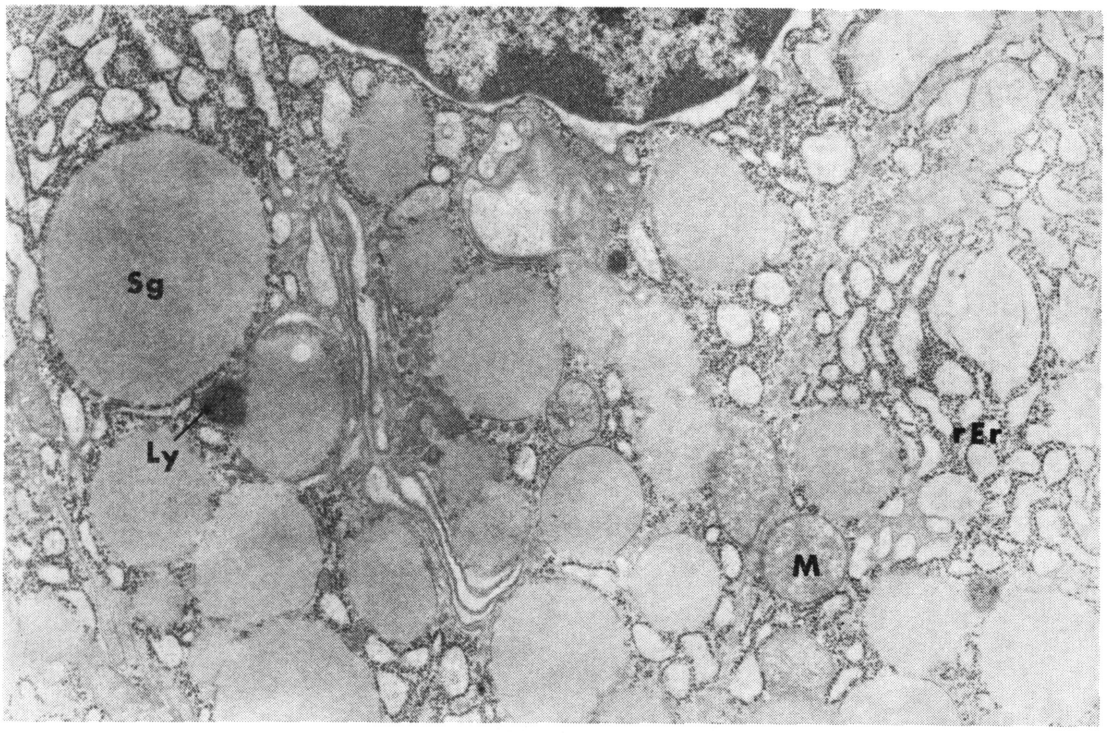

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Photo 7

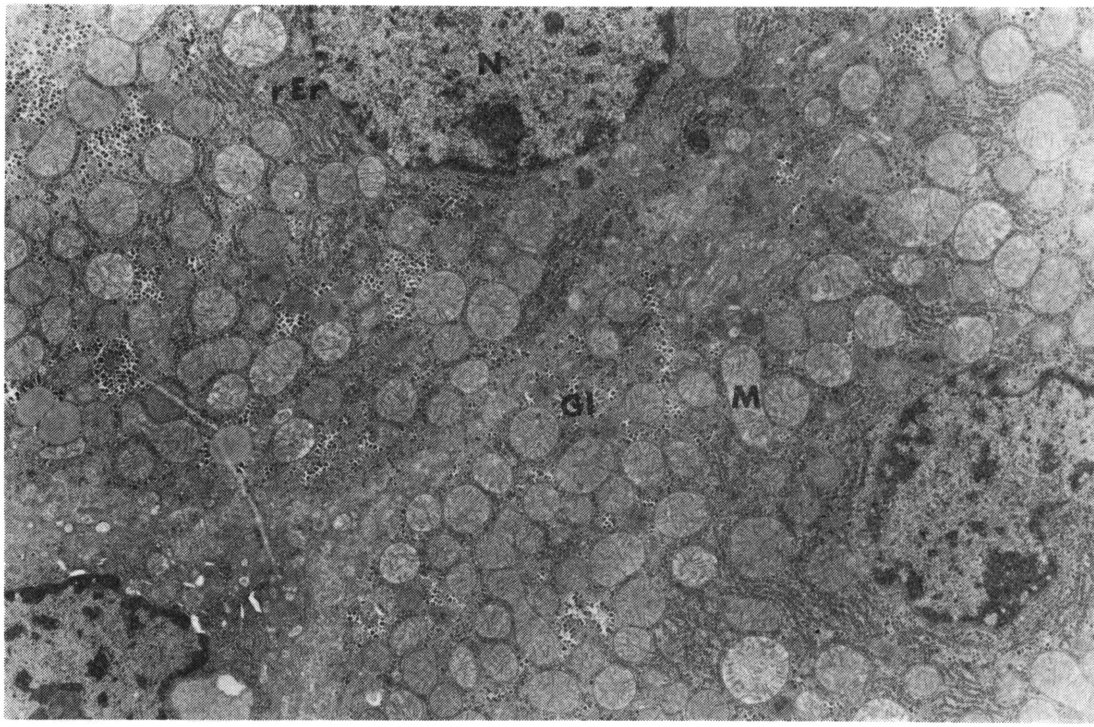


Photo 8

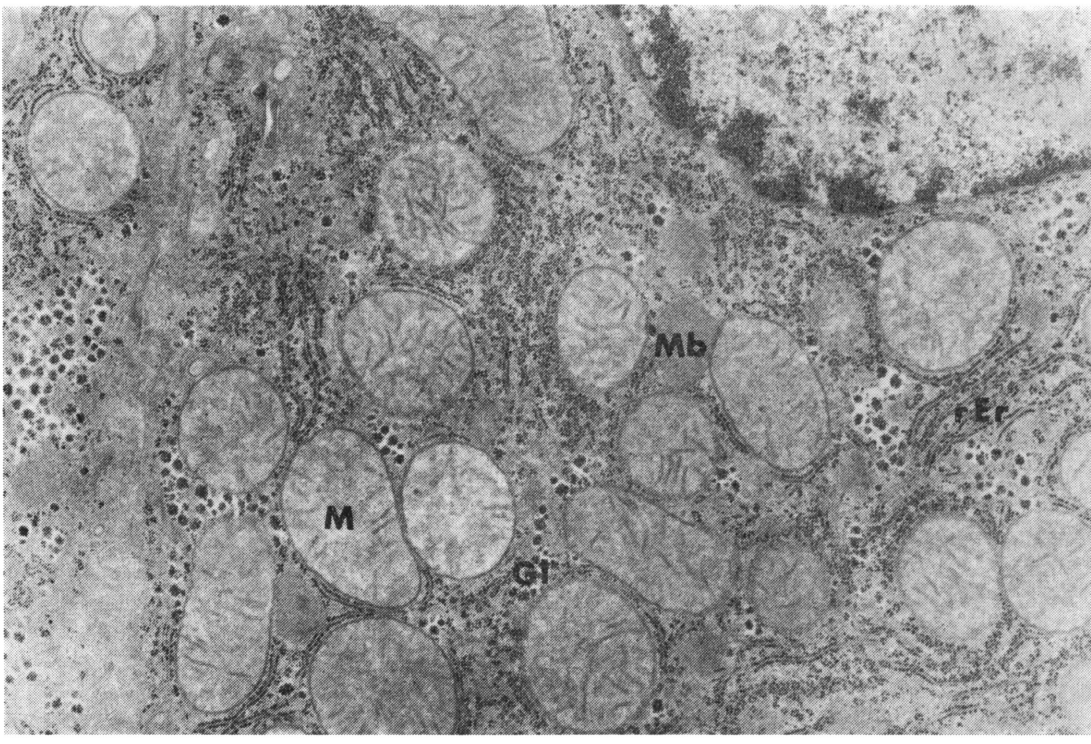


Photo 9

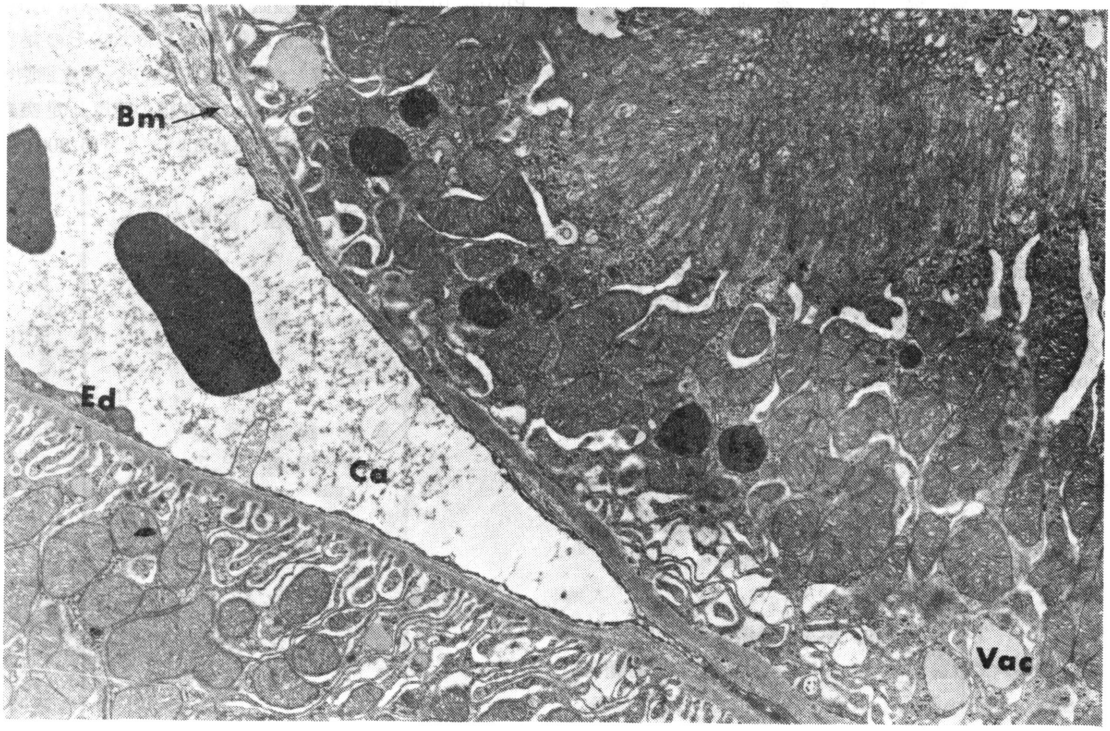
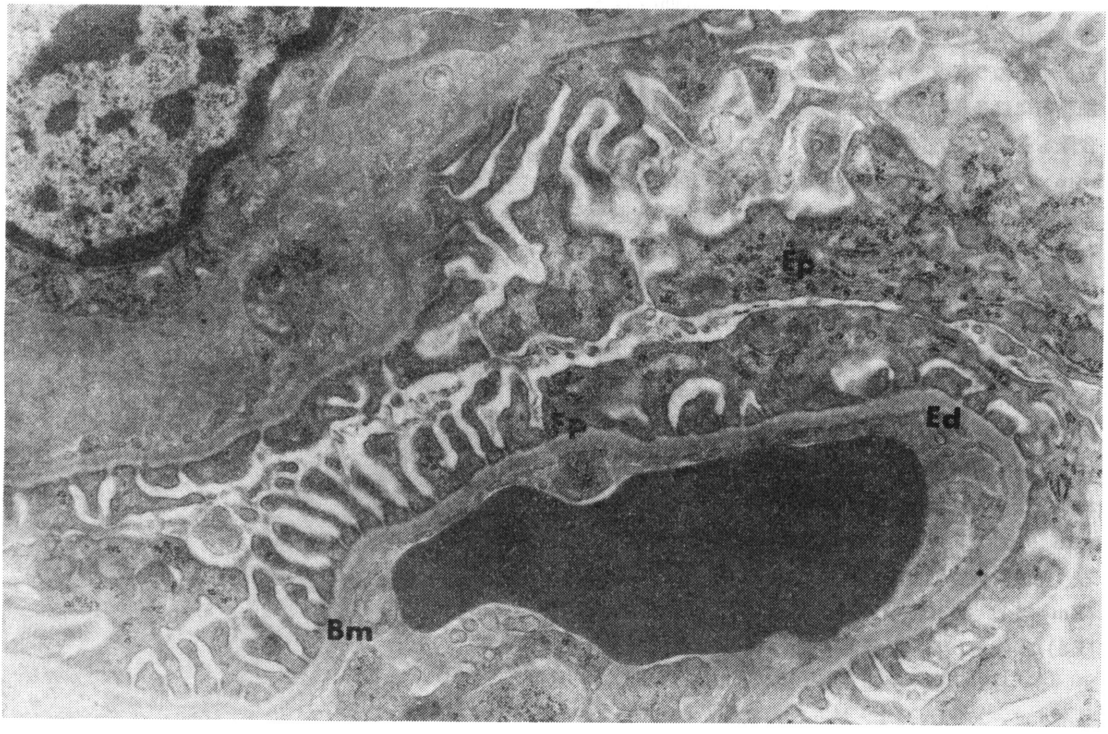


Photo 10



電 顕 写 真 説 明

Photo 1： TFN 500 mg／kg投与の雄の肺所見である。肺胞壁の凹所にみられる大肺胞上皮細胞（Gac）である。肺胞腔に面する自由表面には微絨毛が存在し，細胞内小器官が豊富である。（×15,200）

Photo 2： Photo 1と同様に500 mg／kg投与の雄肺の所見である。肺胞腔（As）は，扁平菲薄な肺胞上皮細胞（Sac）によって囲まれている。毛細血管の内皮細胞（Ed）との間には基底膜（Bm）が介在する。（×15,200）

Photo 3： TFN 500 mg／kg投与の雌脾臓の所見である。リンパ球（L）が多数密集し，その間を縫うようにして細網細胞の突起がみられる。（× 4,750）

Photo 4： Photo 3と同様に500 mg／kg投与の雌脾臓の所見である。リンパ球（L）の他に形質細胞（P）と思われる細胞が多数みられる部分もある。（×4,750）

Photo 5： TFN 500 mg／kg投与の雄胃の所見である。細胞内には，円形ないし楕円形の多数の分泌顆粒（Sg），粗面小胞体（rEr）が発達している。それらの間には糸粒体もみられる。（×5,700）

Photo 6： Photo 5の強拡大である。核周囲には粗面小胞体（rEr）が発達し，糸粒体（M）も豊富である。小数のライソゾーム（Ly）もみられる。また，細胞内には多数の大小の分泌顆粒（Sg）が集積し融合像もみられる。（×15,200）

Photo 7： TFN 500 mg／kg投与の雌肝の所見である。三つの相接する細胞がみられる。粗面小胞体（rEr）は核（N）近傍に平行長紐状に密集し，糸粒体（M）は長円形のものが多い。これらの小器官の間にはグリコーゲン顆粒（Gl）が豊富にみられる。（× 5,700）

Photo 8： Photo 7の強拡大である。胞体内には糸粒体（M），平行配列を示す粗面小胞体（rEr），グリコーゲン顆粒（Gl），microbody（Mb）などがみられる。左下には胆毛細管がある。（×15,200）

Photo 9： TFN 500 mg／kg投与の雌腎臓の所見である。近位尿細胞管上皮の細胞質基底部で，基底膜（Bm）をはさんで間質毛細血管（Ca）と内皮細胞（Ed）がみられる。基底部細胞膜は複雑な陥凹を示している。長い微絨毛突起（刷子縁）と空胞（Vac），ライソゾーム（Ly）などがみられる。（×4,750）

Photo 10： Photo 9の強拡大である。腎糸球体で，毛細血管壁は外側より上皮細胞（Ep）およびその足突起の層（Fp），連続した基底膜（Bm），内皮細胞の薄層（Ed）の3層よりなる。基底膜の肥厚，足突起相互間に特に融合などもみられない。（×15,200）

TABLE 15

Food consumption of male rats receiving TFN (means ± S. D.)

procedure	No. of rats	withdrawal period (days) 0	2	4	6	8	10	12	14
control	5)	22.85	21.50	22.50	23.59	25.41	24.92	25.30	26.00
	±	1.68	2.05	1.64	2.52	1.67	2.43	1.90	1.31
TFN	5)	23.01	22.98	22.89	23.90	24.53	24.63	25.13	25.66
250mg/kg p. o.		1.00	0.94	1.49	1.32	2.01	2.22	1.13	1.72
TFN	5)	21.54	24.17	23.77	23.96	23.84	25.02	25.19	25.48
500mg/kg p. o.		0.87	1.64	2.09	0.92	1.32	2.96	1.84	1.76

food consumption : g

TABLE 16

Food consumption of female rats receiving TFN (means ± S. D.)

procedure	No. of rats	withdrawal period (days) 0	2	4	6	8	10	12	14
control	5)	23.75	23.92	24.01	24.26	23.60	25.39	25.46	25.86
	±	2.40	1.36	1.39	1.95	2.16	2.15	1.77	2.02
TFN	5)	23.69	22.55	23.00	23.92	24.00	24.49	24.92	24.85
250mg/kg p. o.		0.73	1.43	1.84	2.47	3.27	1.95	2.06	1.18
TFN	5)	21.42	21.85	22.84	23.01	22.98	24.63	25.29	24.68
500mg/kg p. o.		1.32	2.25	1.93	2.65	1.52	1.82	2.09	2.78

food consumption : g

TABLE 17

Changes in body weight of male rats receiving TFN

(means ± S. D.)

proceduse	No. of rats	withdrawal period (days) 0	2	4	6	8	10	12	14
control	5)	285.20	299.80	310.20	320.60	337.40	349.40	356.40	363.40
		± 46.75	40.37	40.86	41.66	38.86	35.21	35.91	30.81
TFN	5)	241.25	261.25	273.75	280.75	292.50	297.25	312.50	321.45
250mg/kg p. o.		15.56	13.25	18.45	20.32	25.54	24.05	24.75	21.23
TFN	5)	250.33	263.33	279.33	283.00	296.10	312.15	316.04	323.33
500mg/kg p. o.		21.36	21.57	24.10	25.94	24.56	22.54	24.98	23.86

body weight : g

TABLE 18

Changes in body weight of female rats receiving TFN

(means ± S. D.)

procedure	No. of rats	withdrawal period (days)							
		0	2	4	6	8	10	12	14
control	5)	208.40	217.40	219.20	221.20	224.20	228.40	231.80	246.75
	±	22.81	12.86	13.39	16.21	12.83	16.20	15.17	16.52
TFN	5)	203.75	210.50	216.25	222.00	223.75	229.25	231.25	245.10
250mg/kg p. o.		10.66	14.27	13.12	15.90	17.17	17.39	17.39	15.42
TFN	5)	181.00	188.00	193.33	203.33	209.67	213.00	223.00	231.15
500mg/kg p. o.		15.52	11.14	9.45	7.64	13.87	9.17	10.44	8.64

body weight : g

TABLE 19

Examination of urine

procedure	No. of rats	recovered rats													
		protein			glucose		ketone		bilirubin		benzidine test		urobilinogen	
		(±)	(+)	(++)	(−)	(+)	(−)	(+)	(−)	(+)	(−)	(+)	(±)	(+)
MALE														
control	5	2	3	0	5	0	5	0	5	0	5	0	5	0
250mg／kg p. o.	5	3	2	0	5	0	5	0	5	0	5	0	5	0
500mg／kg p. o.	5	3	2	0	5	0	5	0	5	0	5	0	5	0
FEMALE														
control	5	2	3	0	5	0	5	0	5	0	5	0	5	0
250mg／kg p. o.	5	3	2	0	5	0	5	0	5	0	5	0	5	0
500mg／kg p. o.	5	3	2	0	5	0	5	0	5	0	5	0	5	0

TABLE 20

Hematological findings in male rats during recovery period after treatment with TFN orally for 1 month

	Groups (mean ± S. E.)		
	Control	250mg/kg/day p. o.	500mg/kg/day p. o.
hemoglobin (g/dl)	15.1± 0.5	14.9± 0.5	14.8± 1.3
hematocrit (%)	44.3± 1.7	44.4± 1.2	38.3± 0.8
erythrocytes ($\times 10^4$/mm^3)	696 ±43	685 ±12	717 ±57
leucocytes ($\times 10^2$/mm^3)	101 ± 8	101 ±12	121 ± 5
bosophils (%)	0	0	0
eosinophils (%)	1.0± 0.4	1.1± 0.6	0
lymphocytes (%)	84.8± 2.9	84.6± 2.3	84.0± 4.0
monocytes (%)	1.6± 0.4	1.3± 0.7	0.5± 0.3
neutrophils (%)	12.6± 1.0	13.0± 1.3	15.5± 1.2

TABLE 21

Hematological findings in female rats during recovery period after treatment with TFN orally for 1 month

	Groups (mean ± S. E.)		
	Control	250mg/kg/day p. o.	500mg/kg/day p. o.
hemoglobin (g/dl)	13.2± 0.2	12.9± 0.2	13.0± 0.2
hematocrit (%)	39.2± 1.2	38.8± 0.3	36.8± 0.4
erythrocytes ($\times 10^4$/mm^3)	632 ±32	622 ±34	557 ±24
leucocytes ($\times 10^2$/mm^3)	108 ± 5	106 ± 3	101 ± 7
basophils (%)	0	0	0
eosinophils (%)	0.3± 0.2	0.3± 0.2	0.3± 0.3
lymphocytes (%)	89.2± 1.4	84.8± 2.5	92.0± 0.8
monocytes (%)	1.0± 0.3	1.3± 0.8	0
neutrophils (%)	9.6± 1.1	13.6± 1.6	7.7± 0.7

TABLE 22

Blood Biochemical findings in rats treated orally with TFN for 1 month and kept without treatment of TFN for 2 weeks (No. 1)

procedure	No. of rats	Total Protein (g/dl)	A/G	Glucose (mg/dl)	BUN (mg/dl)	Total Cholesterol (mg/dl)	GOT (K-M unit)	GPT (K-M unit)
MALE								
control	5	5.86±0.12	1.15±0.08	175.2±12.4	19.6±0.8	67.6±2.2	61.6±5.3	27.6±2.6
250mg/kg p. o.	5	5.78±0.23	1.15±0.12	156.7±11.9	19.4±1.3	65.0±4.6	75.0±2.4**	28.0±4.0
500mg/kg p. o.	5	5.80±0.14	1.18±0.02	185.5±18.5	20.8±2.0	67.8±2.7	73.0±3.4*	35.0±2.4*
FEMALE								
control	5	5.82±0.07	1.29±0.09	219.6± 6.9	15.5±0.8	70.9±3.2	63.2±3.3	22.8±2.8
250mg/kg p. o.	5	5.65±0.07	1.29±0.10	212.7± 7.3	16.0±1.1	66.6±2.4	67.8±3.8	25.8±1.7
500mg/kg p. o.	5	5.73±0.13	1.24±0.06	225.0±16.26	19.7±1.9	67.6±4.3	71.7±4.5*	28.0±1.8*

Mean ± S. E.

*: Significant difference from control ($p<0.05$)

**: Significant difference from control ($p<0.01$)

TABLE 23

Blood Biochemical findings in rats treated orally with TFN for 1 month and kept without treatment of TFN for 2 weeks (No. 2)

procedure	No. of rats	ALP (K-A unit)	Creatinine (mg/dl)	Total Bilirubin (mg/dl)	Sodium (mEq/L)	Potassium (mEq/L)	Chloride (mEq/L)
MALE							
control	5	28.9±2.2	0.69±0.04	0.21±0.05	136.0±1.4	3.53±0.34	111.0±1.0
250mg/kg p. o.	5	32.4±2.4	0.66±0.04	0.21±0.06	142.4±5.3	3.89±0.11	113.8±0.9
500mg/kg p. o.	5	39.7±2.1*	0.71±0.02	0.25±0.04	128.4±3.1	3.99±0.24	111.0±0.7
FEMALE							
control	5	28.1±0.9	0.69±0.09	0.23±0.01	132.5±2.4	3.31±0.08	111.6±0.8
250mg/kg p. o.	5	31.6±2.5	0.63±0.08	0.21±0.06	136.6±2.0	3.48±0.16	111.0±0.7
500mg/kg p. o.	5	37.2±3.4*	0.65±0.05	0.23±0.01	140.1±3.2	3.32±0.02	111.3±1.1

Mean ± S. E.

*: Significant difference from control ($p<0.05$)

TABLE 24

Organ weight in male rats treated orally with TFN for 1 month and kept without treatment TFN for 2 weeks

	Control	250mg／kg	500mg／kg
No. of rats	5	5	5
body weight (g)	363.4 ±30.81	321.5 ±21.23	323.3 ±23.86
brain (g)	1.88± 0.100	1.88± 0.132	1.88± 0.071
hypophysis (mg)	10.1 ± 1.85	9.4± 1.40	9.9 ± 0.65
thymus (g)	0.62± 0.139	0.61± 0.235	0.57± 0.077
lung (g)	1.71± 0.146	1.65± 0.138	1.61± 0.189
heart (g)	1.09± 0.108	0.98± 0.159	1.05± 0.052
liver (g)	9.72± 1.848	8.22± 0.422	10.58± 0.718
spleen (g)	0.69± 0.111	0.72± 0.289	0.77± 0.314
kidney (left) (g)	1.13± 0.117	1.08± 0.120	1.03± 0.038
(right) (g)	1.19± 0.141	1.07± 0.154	1.04± 0.076
adrenal (left) (mg)	26.5 ± 5.00	26.4 ± 8.12	25.7 ± 5.81
(right) (mg)	23.7 ± 5.31	25.3 ± 5.11	26.0 ± 7.01
testis (left) (g)	1.53± 0.148	1.49± 0.056	1.48± 0.080
(right) (g)	1.53± 0.275	1.50± 0.047	1.49± 0.083

Results are given means ± S. D.

TABLE 25

Organ weight in female rats treated orally with TFN for 1 month and kept without treatment TFN for 2 weeks

	Control	250mg／kg	500mg／kg
No. of rats	5	5	5
Body weight (g)	246.8 ±16.52	245.1 ±15.42	231.2 ± 8.64
brain (g)	1.86± 0.050	1.84± 0.044	1.84± 0.096
hypophysis (mg)	8.6 ± 1.93	8.8± 3.43	8.4 ± 2.22
thymus (g)	0.69± 0.154	0.58± 0.151	0.60± 0.125
lung (g)	1.62± 0.309	1.62± 0.042	1.62± 0.279
heart (g)	0.86± 0.036	0.86± 0.032	0.83± 0.020
liver (g)	8.20± 0.811	7.59± 0.867	8.58± 0.410
spleen (g)	0.63± 1.00	0.63± 0.194	0.57± 0.041
kidney (left) (g)	0.85± 0.063	0.85± 0.184	0.82± 0.022
(right) (g)	0.86± 0.081	0.85± 0.053	0.81± 0.003
adrenal (left) (mg)	24.6 ± 5.05	26.7 ± 4.33	27.4 ± 6.61
(right) (mg)	24.1 ± 5.24	25.2 ± 3.06	26.4 ± 5.31
Ovary (left) (mg)	75.8 ±17.68	80.7 ±18.01	79.9 ±12.59
(right) (mg)	71.7 ±22.71	79.3 ±15.26	70.4 ± 7.79

Results are given means ± S. D.

TABLE 26

Relative organ weight in male rats treated orally with TFN for 1 month and kept without treatment of TFN for 2 weeks

	Control	250mg／kg	500mg／kg
No. of rats	5	5	5
body weight	363.4 ±30.81	321.5 ±21.23	323.3 ±23.86
brain	0.52± 0.086	0.58± 0.059	0.58± 0.035
hypophysis **	2 80± 0.38	2.90± 0.62	3.10± 0.25
thymus	0.17± 0.039	0.19± 0.096	0.18± 0.039
lung	0.47± 0.098	0.51± 0.135	0.50± 0.063
heart	0.30±0.027	0.30± 0.068	0.32± 0.043
liver	2.68± 0.315	2.57± 0.242	3.28± 0.317*
spleen	0.19± 0.038	0.22± 0.081	0.24± 0.088
kidney (left)	0.32± 0.022	0.34± 0.057	0.32± 0.026
(girht)	0.33± 0.034	0.33± 0.043	0.32± 0.028
adrenal (left) **	7.31± 1.469	8.21± 2.545	7.94± 1.603
(right) **	6.82± 1.396	7.96± 1.184	8.04± 1.668
testis (left)	0.42± 0.047	0.46± 0.029	0.46± 0.018
(right)	0.42± 0.059	0.47± 0.031	0.46± 0.027

Results are given means ± S. D.

Relative organ weight= g／100g body weight **=mg／100g body weight

*: Significant difference from control ($p<0.05$)

TABLE 27

Relative organ weight in female rats treated orally with TFN for 1 month and kept without treatment of TFN for 2 weeks

	Control	250mg／kg	500mg／g
No. of rats	5	5	5
body weight	246.8 ±16.52	245.1±15.42	231.2± 8.64
brain	0.75± 0.045	0.75± 0.054	0.80± 0.059
hypophysis **	3.50± 0.66	3.60± 0.89	3.60± 0.87
thymus	0.28± 0.061	0.24± 0.098	0.26± 0.052
lung	0.66± 0.095	0.66± 0.048	0.71± 0.092
heart	0.35± 0.022	0.35± 0.037	0.36± 0.006
liver	3.32± 0.207	3.24± 0.121	3.71± 0.141*
spleen	0.26± 0.033	0.26± 0.071	0.25± 0.026
kidney (left)	0.34± 0.019	0.35± 0.056	0.35± 0.009
(right)	0.35± 0.020	0.35± 0.040	0.35± 0.001
adrenal (left) **	9.97± 1.719	10.89± 1.091	11.85± 1.901
(right) **	9.76± 2.293	10.38± 1.667	11.43± 1.635
ovary (left) **	30.72± 8.753	32.93±10.217	32.30± 5.020
(right) **	29.06±10.828	32.36± 9.133	32.45± 2.747

Results are given means ± S. D.

Relative organ weight= g／100 g body weight **=mg／100 g body weight

*: Significant difference from control ($p<0.05$)