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OBSERVATIONS ON SOME TISSUES OF MICE INJECTED WITH LAS SYNTHETIC DETERGENT FOR VARYING PERIODS FROM THE DAY OF BIRTH

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Abstract

Effects of chronic exposure to a synthetic detergent (LAS-SD) were studied in mice.

Epilation and dermatitis usually occurred in animals given continued injections of SD. Adhesions between some organs were observed in those receiving injections of SD from the day of birth. Growth, reproduction and survival of the animals were not affected.

Although some organs were increased in relative weight, histopathologic examinations of liver, kidney, adrenals and thyroid organs of treated mice revealed no evidence of poisoning.

INTRODUCTION

As compared with branched-chain alkylbenzene sulfonates (ABS), linear alkylbenzene sulfonates (LAS) are known to be more readily degraded by microorganisms and plants in the soil and sewage. The low toxicity of ABS has been well documented (Coughlin, 1965[1]; Tusing *et al.*, 1960[2]). LAS has also been reported to exert no adverse effects upon growth, survival, hematologic values, urinary analysis, organ weights and histological structure of varying tissues (Kay *et al.*, 1965[3]; Bernard *et al.*, 1965[4]; William *et al.*, 1968[5]).

On the other hand, however, some ill effects of ABS and LAS synthetic detergents have been suggested, e. g. inhibition of hepatic microsomal enzymes (Yanagisawa, 1966[6]), decrease in serum calcium level (Yanagisawa, 1964[7], 1966), hepatic lesions (Sakashita, 1975[8]) and impairment of reproductive activity (Mikami, 1977[9]).

The present experiments were designed to ascertain whether alterations would be caused in structure of various tissues in animals subjected to chronic administration of LAS-SD from the day of birth.

The abbreviations used are: ABS, alkylbenzene sulfonate; LAS, linear alkylbenzene sulfonate; SD, synthetic detergent

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MATERIALS AND METHODS

Male and female mice of the C57 BL/TW strain used in these studies were raised in a temperature (23±1°C)- and light (14h light/day)- controlled room. All animals were allowed to take food and water *ad libitum*.

A LAS-SD on the market (LION YUSHI), was dissolved in distilled water, autoclaved, and injected subcutaneously according to the following schedule: 0.02 ml of 1 % SD for 10 consecutive days from the day of birth (=day 1), 0.04 ml of 1 % SD for the following 10 days (day 11 - day 20), 0.02 ml of 10 % SD 5 times over the next 10 days (day 21 - day 30) and 0.04 ml of 10 % SD every other day during further 30 or 60 days. Control animals received injections of a solution of soap powder (MINASAMA SEKKEN) in a similar manner.

All the animals, unless otherwise mentioned, were sacrificed at the end of the treatment periods.

Tissues were fixed in Bovin's solution after being checked for macroscopic alterations. Sections were stained with hematoxylineosin for light microscopic studies. For electron microscopic examinations, tissues from three male mice of each group were fixed in chilled 3 % buffered glutaraldehyde for 90 minutes, postfixed in 1 % OsO_4 for 90 minutes, dehydrated in graded ethanol and propylene oxide, and embedded in epoxy-resin. Sections were stained with lead citrate and uranyl acetate.

To determine the dose of SD available for the present experiments, a single injection of SD at different concentrations was given on the day of birth to groups of 20 mice. Survivors were kept under observations for 30 days after injection. LD_{50} values were calculated by the method of Lichtfield and Wilcoxon (1949[10]). Body and organ weights were analyzed by the Student's *t* test.

RESULTS

(i) Gross observations on treated animals

Regardless of duration of treatment, epilations occurred in a majority of mice given continued injections of SD, around the site of injections on the dorsal skin. Even a single injection caused epilation or localized discoloration of hairs. After several injections, dermatitis frequently developed in the epilated areas.

However, withdrawal of SD brought about recovery of the damages.

By contrast, injections of soap solution were without effect on the skin. Decrease in body weight, ataxia and diarrhea, suggesting toxic symptoms were not observed.

(ii) Influence of neonatal treatment

When given to mice on the day of birth as a single injection, the LD_{50} was 0.02 ml of 5.9 % SD and the lethal dose, 0.02 ml of 10 % SD. Even if the total dose was equal, the mortality varied with SD concentrations, the mortality following 0.04 ml of 5 % SD reaching 90 (%). Mice treated on day 1 usually died between 6 and 24 hours after injection. Survivors grew well.

In mice injected with SD from the day of birth and sacrificed on day 60 or 90, adhesions sometimes had taken place between different organs. Adhesions never occurred if SD treatment was started on day 11 or later (Table 1). Adhesions between spleen and kidney were frequently encountered (Fig. 1),

TABLE 1.

Visceral adhesions in adult SD-treated mice and controls

Injection	number	%
LAS-SD		
day 1-day60 or 90	165	60.6
day11-day60 or 90	20	0
day31-day60 or 90	10	0
Soap		
day 1-day60 or 90	40	0
Untreated	70	0

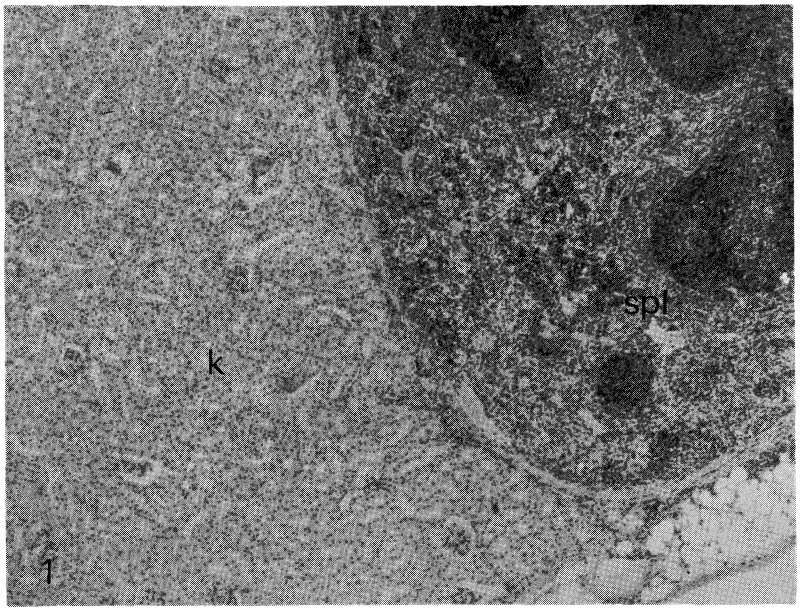

Fig. 1. Photomicrograph showing adhesion between kidney(k) and spleen(spl) in a mouse treated with SD for 60 days. ×160

those between liver and kidney being rare. In soap-treated controls, adhesions were not produced.

(iii) Mean body weight and relative organ weights

In comparison with the untreated and soap-controls, the kidney and liver significantly increased in relative weight after SD treatment for a 60-day period (Table 2, $P < 0.005$). The spleens of female mice, especially of those carrying dermatitis, were markedly enlarged.

However, in male mice, there were no appreciable differences in size of spleen among the three groups (Table 2). The hypertrophy of the kidney and liver, however, gradually declined with advancing age, even if SD injections were continued (Table 3 (a), (b)).

TABLE 2.

Mean body weights and relative organ weights (±SE) in 60-day-old SD-treated and control mice

Number		Mean B.W.	Relative organ weight mg/20g B.W.			
			adrenals	kidney	liver *	spleen
Untreated	18(M)	22.09±0.66	3.75±0.11	278.0±3.59	1.13±0.03	101.8±7.72
	3(F)	17.30±2.38	—	—	1.13±0	129.3±1.90
Soap	8(M)	21.40±0.43	3.85±0.10	277.3±3.40	1.10±0.03	94.3±3.72
	6(F)	18.10±0.52	6.18±0.29	255.7±7.30	0.86±0.17	106.7±5.73
LAS-SD	32(M)	22.97±0.48	3.66±0.22	315.3±4.80	1.24±0.02	105.9±8.53
	12(F)	17.48±0.77	6.09±0.24	302.4±8.42	1.24±0.04	144.7±5.60

* in g/20g B.W.

TABLE 3.

Mean body weights and relative organ weights of SD-treated and control mice at 80 and 90 days of age

(a) 80 days (M)

	number	B.W. (g)	Relative organ weight mg/20g B.W.			
			adrenals	kidney	liver *	spleen
Untreated	14	24.1±0.90	3.53±0.29	290.6±5.11	0.96±0.02	75.9±4.71
LAS-SD	12	25.8±0.44	2.55±0.11	287.8±4.87	1.22±0.03	90.2±3.51

(b) 90 days (F)

	number	B.W. (g)	Relative organ weight mg/20g B.W.			
			adrenals	kidney	liver *	spleen
Untreated	3	21.3±0.23	5.9±0.60	271.6± 2.6	1.13±0.03	129.6±6.12
LAS-SD	4	20.0±1.84	5.4±0.70	293.7±10.9	1.08±0.03	123.0±11.0

* in g/20g B.W.

The adrenals were slightly decreased in weight in the SD-treated animals. The mean body weights of the SD-mice were not significantly different from those of the controls.

(iv) Light- and electron-microscopic observations

At autopsy, the adrenals, liver, kidney, spleen and thyroid of SD-treated mice exhibited neither macroscopic nor light-microscopic changes. Electron-microscopic examinations, also failed to reveal any marked effects in the liver, kidney, adrenals and thyroid.

In hepatocytes, pycnosis, widening of intercellular spaces and degeneration of mitochondria, as reported by Sakashita (1975), were not observed even after long term SD administrations. Smooth-surfaced endoplasmic reticulum was never better developed in SD-mice than in the controls.

In the epithelium of proximal tubules of the kidney of 1 of 3 animals treated with SD for 60 days, nuclei varied considerably in electron density among different cells (Fig. 2). This suggests that an active proliferation of epithelial cells is occurring. Distal tubules and glomeruli were normal in every respect.

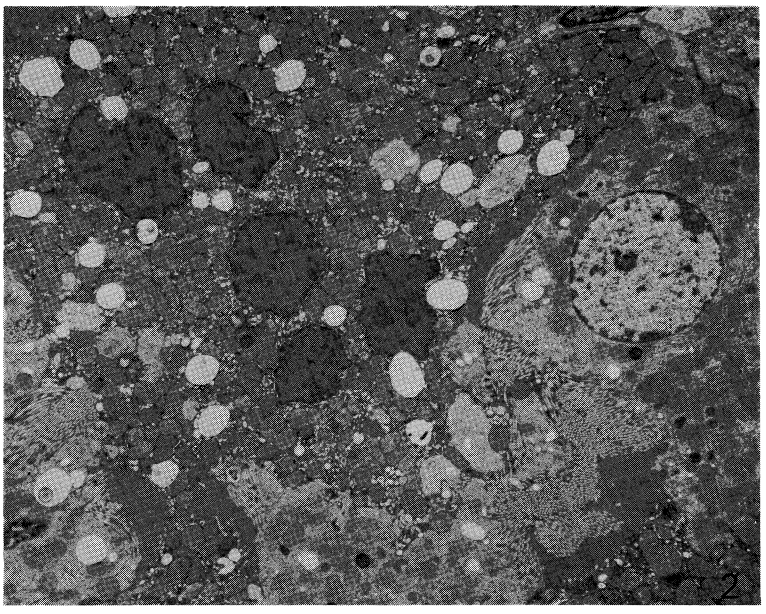

Fig. 2. Part of proximal tubule of kidney from a mouse treated with SD for 60 days, showing some electron-dense nuclei.
×3,070

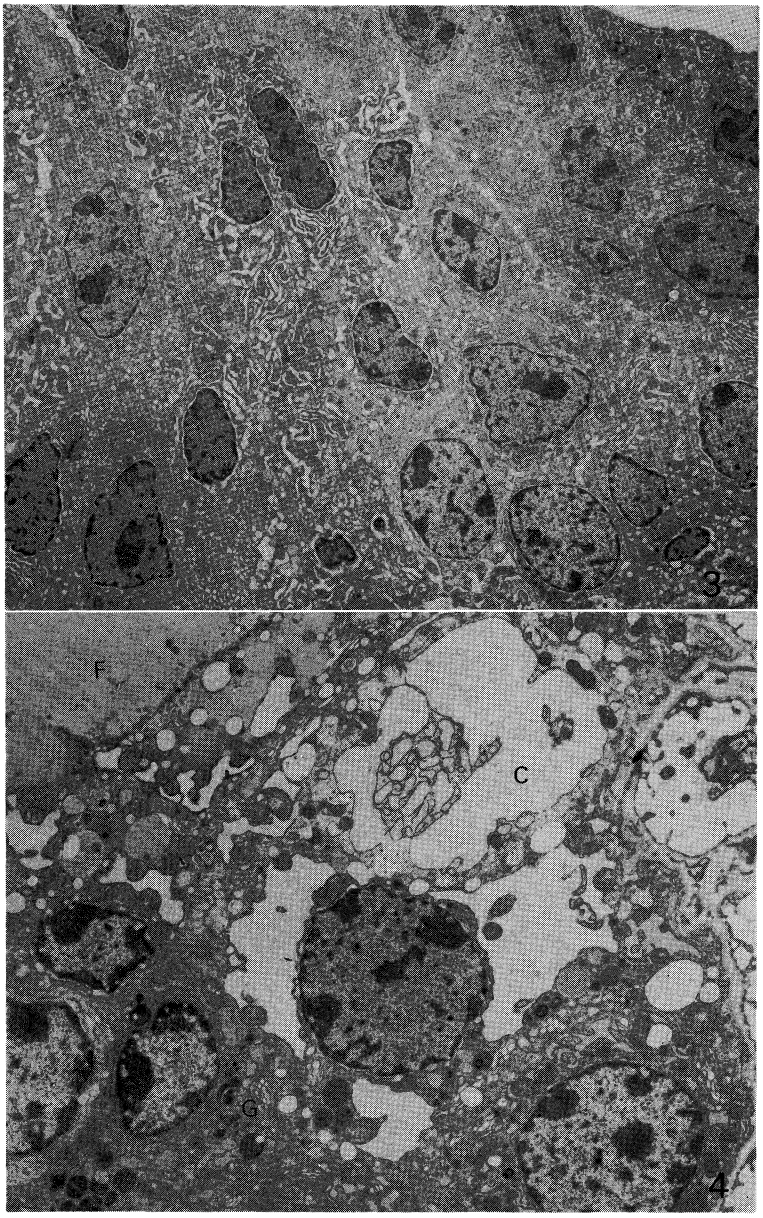

Fig. 3. Low magnification of parathyroid gland from a mouse treated with SD for 60 days. ×3,100

Fig. 4. Thyroid follicullar cells from a male mouse treated with SD for 90 days. Cisternae(c) of RER are considerably distended. F: follicular cavity, G: Golgi complex. ×4,530

Parathyroid glands consisted of chief cells showing different electron density. Rough-surfaced endoplasmic reticulum was well developed with markedly distended cisternae in these cells (Fig. 3).

Thus, the cells appeared to be active in both synthesis and secretion of hormone.

In thyroids, follicular cells were normal in appearance, although rough-surfaced endoplasmic reticulum in these cells had markedly dilated vesicular cisternae (Fig. 4). Parafollicular cells contained numerous secretory granules, some of which were larger than those found in the controls (Fig. 5). Golgi complexes in these cells were poorly developed, like in parafollicular cells of hypocalcaemic animals (Kameda, 1976)[11].

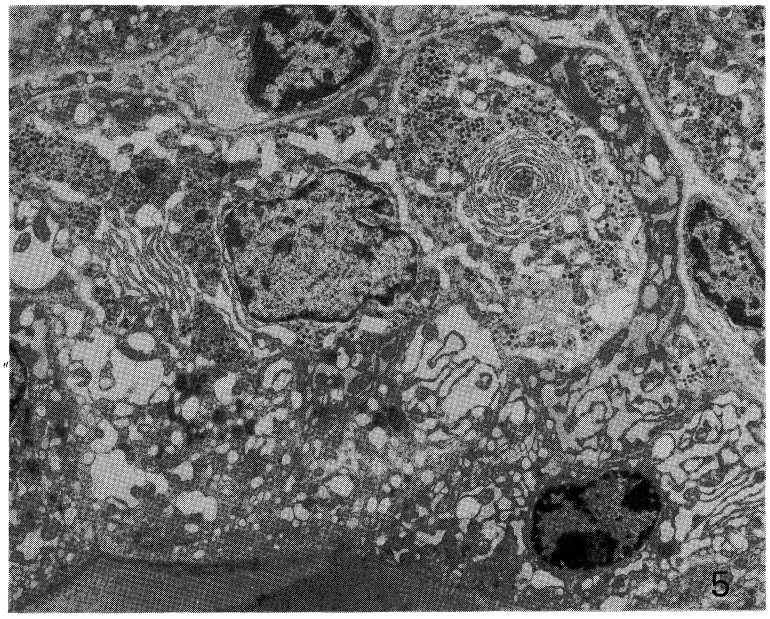

Fig. 5. Thyroid parafollicular cells from a male mouse treated with SD for 90 days. ×4,200

DISCUSSION

Long term administration of large amounts of SD might also result in damages to the skin in other animals including human. Fig. 6 illustrates the skin of an adult rat with severe dermatitis after 5 daily subcutaneous injections of 0.4 ml of undiluted LAS-SD.

In mice given SD on the day of birth, adhesions frequently occurred between some organs (60.6 %). However, they appeared to evoke no harmful

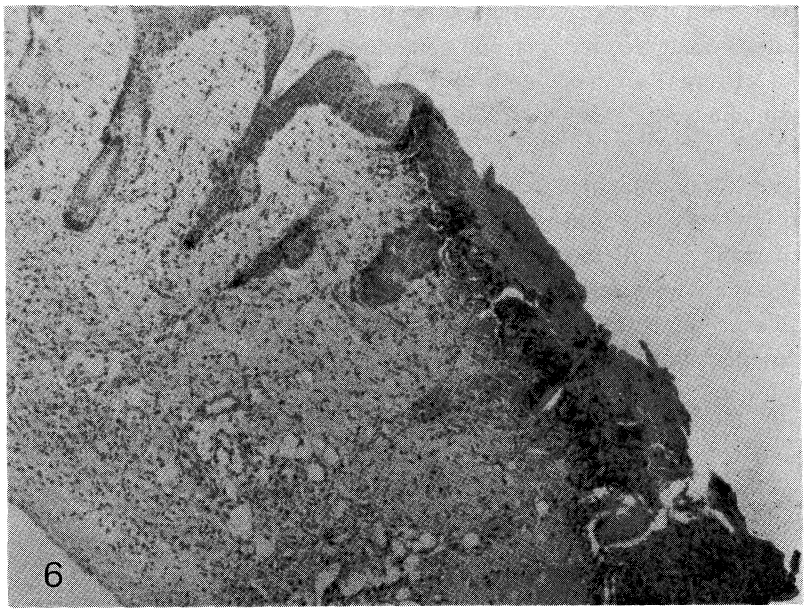

Fig. 6. Skin of an adult rat carrying dermatitis. The rat received SD injections for 5 days. ×160

effects on mice. If produced in the human body, similar adhesions should be troublesome. Light- and electron-microscopic studies on the liver, kidney, adrenals, thyroid and parathyroid of SD-treated mice failed to reveal any marked alterations. The only change which might be ascribable to the effect of SD was found in proximal tubules of the kidney in 1 of 3 male mice (Fig. 2).

Increases in relative weight of the liver and kidney were not accompanied by any microscopic evidence of toxic symptoms.

Parafollicular cells of the thyroid, which are specifically sensitive to changes in concentration of serum calcium, showed features characteristic of hypocalcemia. Consistent with this is the finding of Yanagisawa (1964) that administration of SD caused a decrease in serum calcium level.

Teratoids as reported by Nagai *et al.* (1970[12]) were not found matings between SD-treated male and female mice well fertile. This is in harmony with the data of Buehler (1971[13]).

The present studies appear to show that chronic administrations of LAS synthetic detergent from the day of birth produce no severe injury in varying tisses of recipient mice.

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REFERENCES

1) Coughlin, F. J.: Detergents and water pollution abatement. Am. J. Public Health 55, 760-771, 1965
2) Tusing, T. W., Paynter, O. E. and Opdyke, D. L.: The chronic toxicity of sodium alkylbenzene sulfonte by food and water administration to rats. Toxicol. Appl. Pharmacol. 2, 464-473, 1960
3) Kay, J. H., Kohn, F. E. and Calandra, J. C.: Subacute oral toxicity of a biodegradable, linear alkylbenzene sulfonate. Toxicol. Appl. Pharmacol. 7, 812-818, 1965
4) Bernard, L. O. and Kenneth, M.: Toxicologic studies with branched and linear alkyl benzene sulfonates in rats. Toxicol. Appl. Pharmacol. 7, 819-825, 1965
5) William, R. M.: Metabolism of linear alkylate sulfonate and alkyl benzene sulfonate in albino rats. Toxicol. Appl. Pharmacol. 12, 473-485, 1968
6) Yanagisawa, F.: Jap. J. Clinical & Experimen. Medicine 43, 1504-1509. 1966 (in Japanese)
7) Yanagisawa, F., Watanabe, S. and Yamagishi, T.: Biochemical study on dodecylbenzen sulfonates. (IV) Difference between soft and hard detergents. Jap. J. Public Health 11, 859-864, 1964 (in Japanese)
8) Mikami, Y.: Jap. Medical Journal 2795, 29-30, 1977 (in Japanese)
9) Litchfield, J. T. Jr. and Wilcoxon, F.: A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exptl. Therap. 96, 99-113, 1949
10) Sakashita, S.: Ultrastructural alterations of rat hepatocytes after the administration of various detergents. The Cell 7, 200-211, 1975 (in Japanese)
11) Kameda, Y.: Fine structural and endocrinological aspects of thyroid parafollicular cells. Chromaffin, Enterochromaffin & Related Cells (Chapter 12) 155-170, 1976
12) Nagai, A.: Effects and influences of a detergent upon the development of mouse embryo. Mie Kaibo Gyoseki-shu 18, 61-73, 1970 (in Japanese)
13) Buehler, E. V., Newmann, E. A. and King, W. R.: Two-year feeding and reproduction study in rats with linear alkylbenzene sulfonate (LAS). Toxicol. Appl. Pharmacol. 18, 83-91, 1971