Studies on the Toxic Effects of Mouse Submaxillary Gland Extracts

LEVI-MONTALCINI and COHEN found that the mouse submaxillary gland is a rich source of a protein endowed with a nerve-growth-promoting activity (NGF)[1,2]. Since this important discovery was made, several investigators have studied the biological effects elicited by extracts of this gland in vitro or in vivo. Shortly thereafter COHEN was able to isolate and purify from the same gland another protein factor with growth-promoting activity specific for the epidermal cells (EGF)[3]. Work done more recently in our laboratory has led to the isolation and characterization of a granulocitosis-inducing factor[4] and of a specific hydrolase which induces characteristic changes in cultures of embryonic tissues[5]. In the course of these studies, it was repeatedly observed that crude extracts of the gland are very toxic, even at relatively low protein concentration.

Furthermore, when sublethal doses of these extracts are injected into new-born animals, they elicit a pronounced stunting effect on the growth of these animals. This effect was often observed with partially purified preparation of NGF injected for a prolonged period of time[6]. Atrophy of the lymphoid tissues and thymus involution were observed in the treated animals[7]. Investigations were therefore undertaken to isolate and possibly purify these components of the submaxillary gland extracts responsible for the biological effects mentioned above.

In a first series of experiments, toxicity of gland extracts from adult and prepuberal mice has been investigated.

The results are shown in Table I. As can be seen, on a protein basis, the extracts from male mice are markedly more toxic. Gland extracts taken from animals before puberty are practically non-toxic. Dialysis of these extracts against several changes of *Tris* HCl buffer 50 m*M* pH 7.2 does not result in any appreciable change of the toxic levels. This finding would suggest that the toxic components are associated with macromolecules, possibly of protein nature. The toxic properties of crude extracts are largely destroyed by heating for 10 min at 100 °C. Trypsin digestion (1 h at 37 °C) does not destroy the activity of the extract but causes significant delay of the death of experimental animals.

In an attempt to localize the toxic components, crude extracts of submaxillary glands of male mice were fractionated through Sephadex G 100 column at neutral pH. As can be seen in the Figure, the extract was resolved into a number of distinct components. The fractions of each peak were pooled (5 by 5) and tested for toxicity. With high doses a toxic effect was elicited by all fractions. However, the fractions of the middle part of the chromatogram (pool 2, Figure A) were markedly more toxic than all others. This pool was therefore concentrated by lyophilization and

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Table I. Toxic effect of mouse submaxillary gland extracts injected into new-born and 30-day-old mice

Gland extracts	Dose mg protein/g body weight	Days of treatment	Lethality (%)	Δ% Body weight	Δ% Thymus weight
Male extract	0.1	1	100	–	–
Male extract	0.03	1	50	–	–
Male extract heated 100 °C	0.5	1	0	–	–
Female extract	0.1	1	0	–	–
Female extract	1	1	30	–	–
Female extract	2	1	80	–	–
Prepuber extracts	1	1	0	–	–
Prepuber extracts	5	1	0	–	–
Male extract in new-born mice	0.02	7	40	–35	–80
Male extract in new-born mice	0.01	15	10	–60	–85
Male extract in adult mice	0.01	7	0	–10	–43
Male extract in adult mice	0.01	15	0	–15	–52

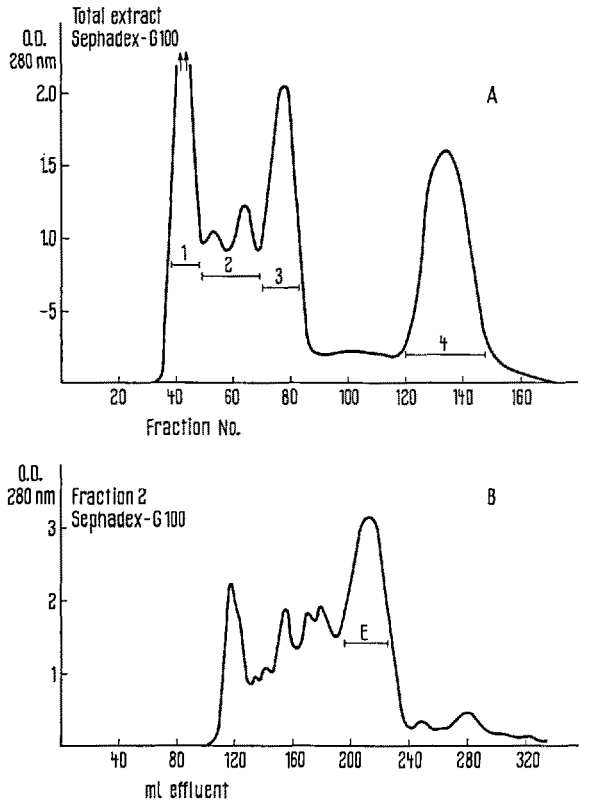
20 experimental and 20 control animals of the same age and sex were used in each experiment. The extracts were injected i.p. in adult mice and s.c. in new-born mice. Changes in body weight and thymus weight of experimental animals are expressed as % of the respective control values.

Table II. Effect of fraction 2 and fraction E in new-born mice

Sample	Dose mg/g body weight	Days of treatment	Lethality (%) of injected animals	Δ% Body weight	Δ% Thymus weight
Pool 2 in new-born mice	0.06	5	100	–	–
Pool 2 in new-born mice	0.04	5	50	–40	–50
Pool E in new-born mice	0.06	6	46	–16	–45
Pool E in new-born mice	0.04	6	10	– 9	–35
Pool E in adult mice	0.04	6	0	– 5	–25

Results expressed as % of the respective control values. 20 experimental and 20 control animals were used in each experiment.

fractionated on a second Sephadex G 100 column. On this column, a pool No. 2 was further resolved into a number of components (Figure B) and each tested for toxicity. The highest level was localized in fraction E (see Figure B). Sublethal doses of this fraction had a moderate stunting effect on the body growth of new-born mice but produced a severe atrophy of the thymus gland (Table II). This effect was very dramatic when the fraction was injected into new-born animals but it was also present in adult animals. No significant effect was found in the spleen or in other control organs. Histological examination of the thymus of treated animals showed a severe atrophy of the follicles and a relative predominance of reticular endothelial cells.



A) Fraction of male mouse submaxillary gland extract on Sephadex G 100 equilibrated with *Tris* HCl buffer 50 m*M*, pH 7.2. Fractions pooled according to the numbers. (B) Fraction of pool 2 from the first Sephadex column on a second G 100 under the same conditions. Fractions from 200–235 ml were pooled together (pool E).

The results of these experiments indicate that, in the crude extract of the submaxillary gland, there are several components responsible for a generalized toxic and stunting effect when injected into mice. These components, some of which appear to be proteins, can be separated and individually characterized. Fraction E is lethal at high doses, but at low doses the toxic effect is restricted to one target organ, namely the thymus. Preliminary experiments indicate that such effect is also elicited in vitro on dissociated thymocytes. Further purification of these fractions and characterization of their toxic effect on the thymus in now in progress[8].

Riassunto. È stata studiata l'azione tossica di estratti di ghiandola sottomascellare di topo. La tossicità dell'estratto totale appare la risultante di varie componenti alcune delle quali di natura proteica. Mediante gel-filtrazione, è stata isolata e parzialmente purificata una frazione che a dosi subletali provoca una marcata atrofia del timo in topolini neonati ed adulti.

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The Effect of Diasone® Sodium on the Iodide Uptake in the Rat Thyroid

Pittman et al.[1] did not find that the sulfone Sulfoxone (Diasone® sodium: 4, 4′-diaminodiphenylsulfone disodium formaldehyde sulfoxylate) had any effect on the thyroid of young rats. Experiments undertaken by the author in order to examine the effect of the drug on the rat thyroid gland have, however, given divergent results. In the present report the inhibition of the in vivo uptake of sodium iodide-^{125}I in rat thyroid and the in vitro inhibition of iodide oxidation by a preparation of Diasone® sodium are described.

Experimental. Male rats of the Sprague-Dawley strain were maintained on a practical type of diet which assured an adequate supply of iodine. At the beginning of the experiment the animals were 26 days old. They were divided at random into a control group and an experimental group, both of which contained 10 animals. The body weights of the animals were approximately the same in the 2 groups, viz. about 70 g at the beginning and about 90 g at the end of the experiment. The rats in the experimental group were injected s.c. for 3 consecutive days with 0.7 ml of a solution of Diasone® sodium (40 mg of the preparation/ml of isotonic saline). This solution was prepared immediately before use, the drug having been kept under vacuum. However, on the fourth day the animals were given 0.9 ml of the Diasone® solution. The animals of the control group were injected with equivalent volumes of isotonic saline. 1 h after the last injection the animals of the 2 groups were injected i.p. with 2.9 μCi of carrier-free sodium iodide-^{125}I in isotonic solution, buffered to pH 7 and stabilized with sodium thiosulphate. The animals were sacrificed 4 h later. The thyroid glands were removed and placed in 2*M* KOH and digested to completeness at 80 °C. After the digestion, the solution in the test-tubes was adjusted to a volume of 3.0 ml. The measurements of the radio-isotope in the digested thyroid glands and that of a diluted standard solution were made with a well-type scintillation counter (crystal: sodium iodide activated with thallium). No countings fell below 13,000 cpm.

To test if Diasone® sodium worked as an inhibitor of the thyroid iodide-peroxidase, some of the manometric

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