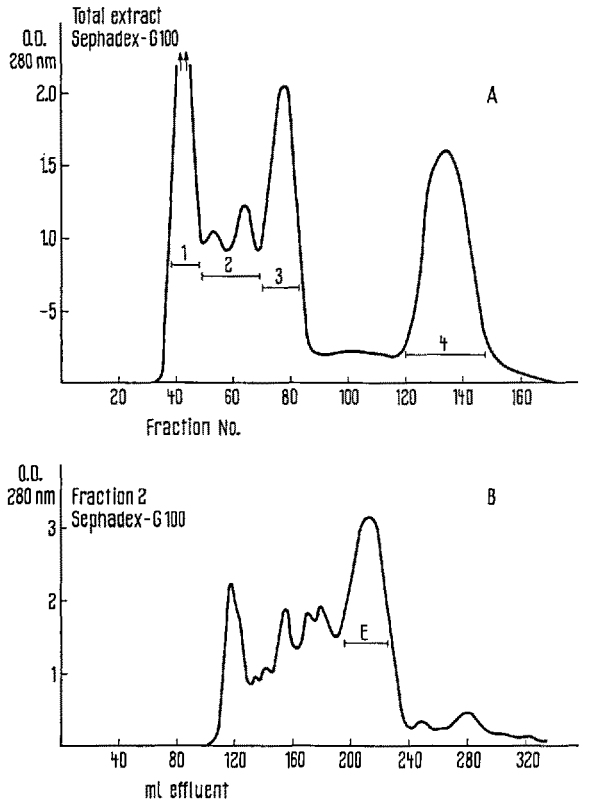
fractionated on a second Sephadex G 100 column. On this column, a pool No. 2 was further resolved into a number of components (Figure B) and each tested for toxicity. The highest level was localized in fraction E (see Figure B). Sublethal doses of this fraction had a moderate stunting effect on the body 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.

A. Liuzzi and P. U. Angeletti

Laboratorio Chimica Biologica, Centro di Neurobiologia, Istituto Superiore di Sanità, Roma (Italy),
24 May 1968.

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

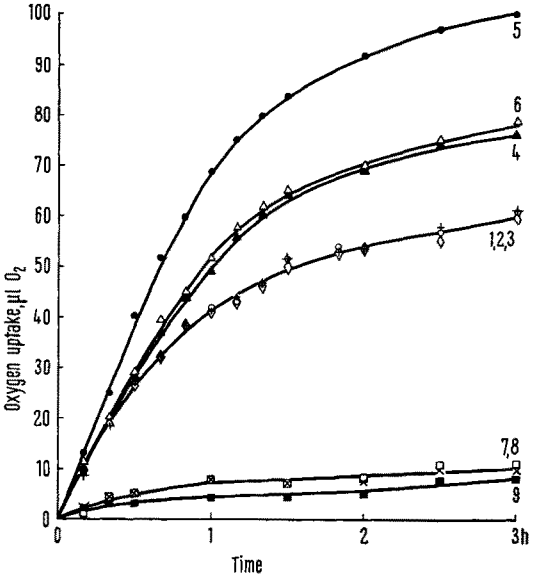
Pittman et al.[1] did not find that the sulfone Sulfoxone (Diasone® sodium: 4, 4′-diaminodiphenylsulfone disodium formaldehyde sulfoxylate) had any effect on the thyroid of young rats. Experiments undertaken by the author in order to examine the effect of the drug on the rat thyroid gland have, however, 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

[1] J. A Pittman, R. W. Brown and W. E. Martindale, Proc. Soc. exp. Biol. Med. *105*, 435 (1960).

procedures described by ALEXANDER[2] were used. In his experiments the hydrogen peroxide which is used in the enzymatic oxidation of iodide is enzymatically generated by glucose oxidase, a process which, among other things, consumes oxygen. The present investigation was carried out in 3 separate steps (legend of Figure). The oxygen consumption was determined by the Warburg constant-volume method at 37 °C, CO_2 being removed by 0.10 ml of 1 *M* KOH on filter-paper wicks in the central cup of the vessel. The reaction mixture in the main compartment contained 0.20 ml of 0.75 *M* phosphate buffer (pH 7.3) and 0.10 ml of 0.050 *M* glucose. In experiment 1, 0.60 ml of isotonic KCl, 0.10 ml of 0.25 *M* freshly prepared KI, and 0.20 ml of a freshly prepared solution of the Diasone® sodium preparation (0.0078 g/ml) were added, when indicated (legend of Figure). After 10 min temperature equilibration 0.10 ml of a solution of glucose oxidase (600 μg/ml, Sigma type II; 1 mg of glucose oxidase will oxidize 14 μM glucose to gluconic acid per min at 35 °C at pH 5.1) was added from one of the side-arms. The final volume of liquid in the main compartment was 1.40 ml, and the gas phase air. The volume of the vessels varied between 12 and 15 ml. The conditions of experiment 2 were the same as those of experiment 1. However, a 5% thyroid homogenate prepared in isotonic KCl was added instead of KCl. The homogenates were centrifuged for 20 min at 1500 *g* to remove tissue fragments. Thyroids from 17–19 adult Wistar rats were used in each experiment. The conditions of experiment 3 were also the same as those of experiment 1. However, glucose oxidase was omitted and glucose, KI, and Diasone® sodium were added from the side arms after temperature equilibration. As in experiment 2, a 5% thyroid homogenate was used. In experiment 2 a check of the presence of iodine was made at the end of the experiment. After addition of trichloroacetic acid and starch solution, the absorption of the iodine starch complex was measured at 570 nm.



Determination of oxygen uptake in experiments 1, 2 and 3. All incubation mixtures contained phosphate buffer, glucose and the following additions. Experiment 1: curve 1, glucose oxidase + KCl; curve 2, glucose oxidase + KCl + Diasone® sodium; curve 3, glucose oxidase + KCl + KI. Experiment 2: curve 4, glucose oxidase + homogenate in KCl; curve 5, glucose oxidase + homogenate in KCl + KI; curve 6, glucose oxidase + homogenate in KCl + KI + Diasone® sodium. Experiment 3: curve 7, homogenate in KCl; curve 8, homogenate in KCl + Diasone® sodium; curve 9, homogenate in KCl + KI. Each point on the curves corresponds to the mean value of 2 measurements.

Result and discussion. The amounts of injected isotope which were taken up by the thyroid glands of the 2 groups are presented as percentages in the Table. As is evident, there is a 44% reduction of the iodide uptake in the experimental group under the prevailing conditions. Regarding the manometric experiments, it is obvious from curves 1, 2, and 3 of the Figure that no spontaneous oxidation of KI by the enzymatically generated H_2O_2 took place. Nor was the glucose oxidase inhibited by Diasone® sodium. In the presence of iodide the oxygen consumption of a sample consisting of the peroxide-generating system and the homogenate was markedly elevated. In the presence of Diasone® sodium this elevation was, however, almost completely eliminated (see curves 4, 5 and 6). The increase in the oxygen consumption and its depression are coincident with a positive and a negative starch test for iodine, respectively. From curves 7, 8 and 9 it follows that a 5% homogenate shows a slight oxygen consumption, and that this consumption is not greatly influenced by Diasone® sodium. To judge from spectral analyses between 225 and 1000 nm no reaction occurs between Diasone® sodium and I^-.

The reduced uptake of iodide under the influence of the Diasone® sodium preparation may be due to different factors, such as an impaired absorption of iodide from the peritoneal cavity, an increased renal excretion of iodide or a direct or indirect effect on the thyroid gland. The manometric experiments support the third factor, indicating that the drug probably inhibits the peroxidase activity.

Regarding the human thyroid gland, it is uncertain if the above-mentioned findings are of clinical interest. However, the sulfone drug may exert an effect on peripheral peroxidases[3].

The influence of Diasone® sodium on the uptake of iodide-^{125}I in the thyroids of rats

Control group	Group treated with Diasone® sodium
1.22 ± 0.25 (10)	0.68 ± 0.16 (10)

Uptake of iodide expressed as a percentage of the injected dose. Mean values with standard deviations are given. No. of animals in brackets.

Zusammenfassung. Diasone® Sodium hemmt die Aufnahme von Radiojod in der Schilddrüse der Ratte. Diese Wirkung entsteht wahrscheinlich durch Hemmung der Jodid-Peroxydase.

H.-O. KARLSSON with the technical assistance of H. ERIKSSON

Institute of Zoophysiology, University of Uppsala (Sweden), 10 May 1968.

[2] N. M. ALEXANDER, J. biol. Chem. *234*, 1530 (1959).

[3] The author wishes to express his gratitude to Prof. P. E. LINDAHL for valuable criticism of the manuscript. Diasone® sodium was kindly supplied by the Abbott Laboratories, USA.