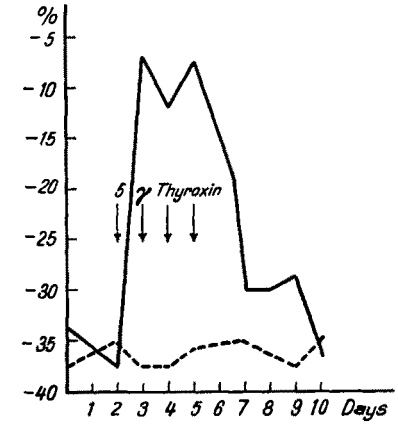
The Effect of Thyroxin in Thyroidectomized Rats Treated with Methylthiouracyl

According to the generally accepted view, thiourea, thiouracyl, and similar drugs act by inhibiting the synthesis of thyroxin in the thyroid gland[1]. This view is based on the experimental fact that several investigators found the response to thyroxin unchanged in animals treated with thiouracyl, and is supported by experiments showing that after the administration of iodine practically no organic iodine can be demonstrated in the thyroid gland of rats treated with thiouracyl, and that thyroid slices *in vitro* do not form organic iodine under the effect of goitrogenous drugs[2]. Accepting this view it must be inferred that the administration of 0·05–0·10 g per kg methylthiouracyl suppresses thyroxin formation completely, for such doses depress within 2–3 weeks the rate of basal metabolism – as our experiments confirm – to the same level as complete thyroidectomy. ABELIN[3] raised some doubts about the validity of this theory. In experiments on normal and thyroidectomized rats he found that the effect of repeated doses of 0·2–1·0 mg thyroxin was smaller in animals treated with methylthiouracyl. Experiments on thyroidectomized animals carry necessarily the most weight, for any action on the thyroid gland can be excluded. ABELIN's results are indicative, but not conclusive. The total doses of thyroxin were exceedingly large (5·0–10·5 mg), and one of three thyroidectomized animals responded with a similar rise of O_2-consumption to that of the untreated controls. Similar doubts regarding the exclusive action of antithyroid drugs on the synthesis of thyroxin were expressed by POUPA[4]. RAWSON and coworkers[5] observed that the action of thyroid administered to cases of myxœdema is diminished by thiouracyl.

Attention was drawn in an earlier communication to the high thyroxin sensitivity of thyroidectomized animals[6]. It could be pointed out that doses more than a thousand times smaller than those employed, for instance, by ABELIN are followed by a marked rise of O_2-consumption in the thyroidectomized rat. It seemed therefore worth while to investigate the problem of the so-called peripheral action of thiouracyl with such small doses of thyroxin. One advantage of these small doses is that results can be excepted to be more clear cut, and the other that they are probably within the range produced by the thyroid glands of normal animals[7].

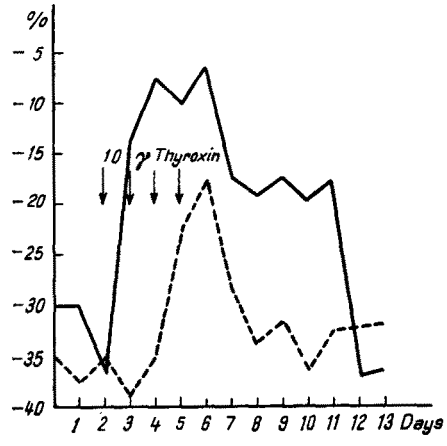
Methods. Male rats of approximately 250 g body weight, accustomed to the procedure of estimating O_2-consumption in the somewhat modified apparatus of BELÁK and ILLÉNYI[1], were used. Thyroidectomy was performed two months earlier, and the administration of 0·10 g per kg methylthiouracyl daily through stomach tube was started four weeks before thyroxin administration. O_2-consumption was measured daily after a fast of approximately eight hours. Thyroxin (Schering) was injected subcutaneously. The experiments were performed during the summer.



——— thyroidectomized — — — — thyroidectomized receiving methylthiouracyl

Fig. 1.

Fig. 1 demonstrates clearly that 5 micrograms of thyroxin elevate O_2-consumption considerably within 24 hours in the untreated thyroidectomized rat, while the rate of metabolism of animals treated with methylthiouracyl remains unchanged even after they have received similar doses for four days. Identical results were obtained with the administration of 1 microgram through seven days and 2 micrograms daily for five



——— thyroidectomized — — — — thyroidectomized receiving methylthiouracyl

Fig. 2.

days, O_2-consumption of the untreated thyroidectomized rat rising after the second dose of 1 and after the first dose of 2 micrograms from –35 and –40 per cent to –20 per cent and maintaining this level throughout the course of thyroxin administration, while the rate of

[1] E. B. ASTWOOD, Harvey Lectures, Series XV (1944–45). – L. S. GARGILL and M. F. LESSES, New England J. Med. *235*, 717 (1946). – J. THYSSEN, Act. pharmacol. et toxicol. *3*, Suppl. 2 (1947).

[2] A. L. FRANKLIN and I. L. CHAIKOFF, J. Biol. Chem. *152*, 294 (1944); cit.: Biol. Abstr. *18*, 13129 (1944). – A. TAUROG, I. L. CHAIKOFF, and D. D. FELLER, J. Biol. Chem. *171*, 189 (1947). – A. L. FRANKLIN, I. L. CHAIKOFF, and S. R. LENNER, J. Biol. Chem. *153*, 151 (1944); cit.: Biol. Abstr. *18*, 15924 (1944).

[3] I. ABELIN, Arch. Int. Pharmacodyn. Thérap. *75*, 187 (1947).

[4] O. POUPA, Sbornik Lekarsky *48*, 219 (1946); cit. Excerpta. med. III., *1*., 13.

[5] R. W. RAWSON, R. D. EVANS, J. H. MEANS, W. C. PEACOCK, J. LERMAN, and R. E. CORTELL, J. Clin. Endocrin. *4*, 1 (1944); cit.: THYSSEN, l. c.

[6] I. ANDIK, L. BALOGH, SZ. DONHOFFER, and GY. MESTYÁN, Exper. *5*, 211 (1949).

[7] W. HURST and C. W. TURNER, Amer. J. Physiol. *150*, 686 (1947). – A. TAUROG and I. L. CHAIKOFF, J. Biol. Chem. *169*, 49 (1947).

[1] S. BELÁK and A. ILLÉNYI, Biochem. Z. *281*, 27 (1935).

metabolism of the animals receiving methylthiouracyl remained absolutely unchanged.

The daily injection of 10 micrograms increases also O_2-consumption of thiouracyl-treated animals, yet, while in the untreated thyroidectomized rat the rate of metabolism rises within 24 hours after the first thyroxin injection and remains elevated for 6 days following the termination of thyroxin administration, O_2-consumption of thiouracyl-treated animals increases only after the third dose of thyroxin and approximates the original level days before the rate of metabolism of the untreated animal returns to its initial value.

Conclusions. It could be demonstrated that methylthiouracyl inhibits the action of small doses of thyroxin completely in the thyroidectomized animal. Therefore it is evident that its action cannot be limited to suspending the synthesis of thyroxin in the thyroid gland, and it must be assumed that at least it inhibits the action of thyroxin in other tissues as well. Considering that very probably the amounts of thyroxin employed in these experiments fall within the range produced by a normal and possibly even by a hyperactive thyroid gland, the question arises whether it is absolutely necessary to assume that the principal pharmacological action of antithyroid drugs is the inhibition of thyroxin synthesis. The most direct evidence in favour of the inhibition of thyroxin synthesis was furnished by CHAIKOFF and coworkers (l. c.) in experiments demonstrating that antithyroid drugs inhibit the formation of organic iodine compounds by thyroid slices *in vitro*. The fact that thyroids of thiouracyl-treated animals are poor in iodine, retain iodine only for a short time, and do not contain organic iodine after administration of iodine carries much less weight, for essentially similar findings can be obtained in hyperactive glands. The experiments reported of course do not disprove the inhibition of thyroxin synthesis by antithyroid drugs, yet they demonstrate clearly the existence of another mechanism capable of accounting even in the presence of physiological amounts of thyroxin for the production of a state of hypothyroidism by antithyroid drugs. The assumption of a fundamentally different action of thiouracyl on the thyroid (inhibition of thyroxin synthesis) and other tissues (inhibition of the action of thyroxin) is rather unsatisfactory, and probably further investigation will furnish an explanation based on a single mechanism. In any case the inhibiting action of methylthiouracyl on the action of thyroxin cannot be disregarded in future attempts to analyse the action of this and similar drugs.

I. ANDIK, L. BALOGH, and Sz. DONHOFFER

Institute of Experimental Pathology and Medical Clinic, University of Pécs, January 20, 1949.

Zusammenfassung

Kleine Thyroxingaben (1–5 μg pro Tag), die den Stoffwechsel schilddrüsenloser Ratten bedeutend erhöhen, sind bei Tieren, die mit Methylthiouracil behandelt wurden, völlig unwirksam. 10 μg täglich verursachen auch im thiouracilbehandelten Tier eine Steigerung des Sauerstoffverbrauches. Dessen Höhe und Dauer bleibt aber weit hinter der eines unbehandelten Tieres zurück. Es wird damit bewiesen, daß Thiouracil *peripher* Thyroxindosen von physiologischer Größenordnung vollkommen unwirksam macht. Es erscheint wenig wahrscheinlich, daß der Effekt des Thiouracils auf zwei prinzipiell verschiedenen Mechanismen (Störung der Thyroxinsynthese in der Schilddrüse und Aufhebung der Thyroxinwirkung in anderen Geweben), beruht. Der Antagonismus Thiouracil: Thyroxin darf jedenfalls bei weiteren Untersuchungen über den Wirkungsmechanismus dieser und ähnlicher Stoffe nicht außer Betracht gelassen werden.

Action of Podophyllin on the Number of Blood Leukocytes

It is well known that the action of caryoclastic DUSTIN[1] or mitotic LUDFORD[2] poisons on white blood-cells count. Colchicine brings about transient leukopenia followed by leukocytosis in the dog and the rabbit DIXON and MALDEN[3] and high leukocytosis in mice LITS[4]. Urethane produces strong diminution of the white blood-cells which counts in human leukemia, although in the normal human PATERSON *et al.*[5] and in the rabbit MOESCHLIN[6] the leukopenic effect is either inconstant or cannot be observed. The fact that podophyllin (KAPLAN[7]) as well as colchicine (KING and SULLIVAN[8]) inhibit cell proliferation in *Condylomata acuminata*, and normal skin suggested that podophyllin could induce modifications in the white blood-cells formation as colchicine does. As it is shown by the experiments reported below, this was actually the case.

White, normal, adult rats, of both sexes weighing 125–245 g, were injected with podophyllin intraperitoneally, first 0·25 mg, and later 0·50 mg each two or three days. The podophyllin was dissolved (0·50 mg/ml) in a 10% v/v ethanol-water mixture. Subcutaneous injections were discarded as a local necrosis was formed at the injection spot.

As reported in Tables I and II a marked diminution ranging between 38–42 p. c. in the number of white blood-cells was always found after the injection of a total dosis of 10·5 mg of podophyllin distributed over 60–75 days. The rats were apparently in good health, increased in weight and never had diarrhea. When distributed over a shorter time (40 days) the same amount of podophyllin produced a 50% leukopenia, but there appeared symptoms of poisoning, like diarrhea loss of weight, and finally death.

We are indebted to the Abbott Laboratories for a generous gift of the podophyllin employed.

Table I

	Days after beginning of injections						
	0	8	20	36	48	60	75
Rat No. 1		9·4	9·1	9·1	9·0	7·5	5·4
2		9·1	8·3	8·0	8·3	7·1	6·2
3		9·6	9·6	9·0	7·8	6·2	6·1
4		9·1	8·4	8·4	8·1	7·3	5·9
Leukocytes × 1,000 (average)	9·5	9·3	8·8	8·6	8·3	7·0	5·9
Total podophyllin injected: 10·50 mg per rat							

[1] A. P. DUSTIN, Le Sang *12*, 677 (1938).
[2] R. J. LUDFORD, Arch. Zellforsch *18*, 411 (1936), cit. by KING and SULLIVAN.
[3] W. E. DIXON and W. MALDEN, J. Physiol. *37*, 50 (1908).
[4] F. J. LITS, Arch. Int. Med. Expérim. *11*, 811 (1936).
[5] E. PATERSON, A. HADDOW, I. THOMAS, and J. M. WATKINSON, Lancet *250*, 677 (1946).
[6] S. MOESCHLIN, Exper. *3*, 195 (1947).
[7] I. W. KAPLAN, New Orleans Med. Surg. J. *94*, 388 (1942).
[8] L. S. KING and M. SULLIVAN, Science *104*, 244 (1946).