the dye occurs at a concentration of the solvent characteristic of the respective substance.

It would be of general interest to investigate whether the effect of various polar solvents on the metachromatic reaction of a chemically defined chromotrope substance could be correlated with any known physicochemical properties of the solvent.

In order to test these hypotheses the following experiments were devised:—

Chromotrope substance:– Water solutions of the sodium salts of chondroitin sulfuric acid[1], hyaluronic acid[1], and heparin were used. Each solution had a concentration of 200 mg p. 100 ml. The heparin used had a strength of 80 Toronto units per mg substance.

Dye:—Toluidine blue 0·4 mg p. 100 ml in water.

Serial dilutions of the chromotrope substances with concentrations ranging from 200 mg p. 100 ml to 12.5 mg p. 100 ml were made. To one ml of each dilution of the different substances was added 9 ml of the solution of the stain.

A purple, metachromatic colour reaction took place in all the concentrations of the chromotrope substances. In no case was there any precipitation.

The following solvents were then added to these test solutions: dioxan, ethanol, methanol, isopropyl alcohol, and acetone until the purple colour completely disappeared and changed to a blue as compared to a standard solution of toluidine blue in water (9 ml dye solution, 0.4 mg p. 100 ml and one ml water).

Results:—With this very crude method it was found that the required amount of the same solvent was relatively constant for the same chromotrope substance, despite the varying concentrations of the latter. However, different amounts of a certain solvent were required by the three different chromotrope substances (see Tables I and II).

Experimental data from a typical experiment given thus would seem to imply that the amount of each polar solvent required to annul the metachromatic reaction between the dye and the various chromotrope substances is not the same. Furthermore, the calculated dielectric increments given in the Table II suggest the possibility that *the dielectric change required to alter the metachromatic bond is of the same order of magnitude for a specific substance.*

Spectrophotometric analyses and measurements of dielectric constants in relation to the phenomenon described above, as well as staining experiments on tissue sections, are in progress.

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Table I

Dielectric increments of polar solvents used (for 1 molar, aqueous solutions). According to the literature.

Solvent	Dielectric increment
Dioxan	−8·3
Ethanol	−2·6
Methanol	−1·4
Isopropyl alcohol	−4·3
Acetone	−3·2

Table II

Dielectric increment required to annul the metachromatic reaction. Calculated from the concentration of the solvents in the test solution.

Chromotrope substance Sodium salts of:	Dioxan	Ethanol	Methanol	Isopropyl alcohol	Acetone
Chondroitin sulphuric acid	−13·7	−10·6	− 8·9	−10·8	− 9·0
Hyaluronic acid	− 8·3	− 7·8	− 7·9	− 8·6	− 7·7
Heparin	−24·2	−20·1	−18·0	−18·8	−20·0

[1] Obtained by courtesy of Prof. G. Blix, Institute of Medical Chemistry, Uppsala.

Zusammenfassung

Gewisse basische Anilinfarbstoffe geben im Bindegewebe eine metachromatische Farbreaktion, welche jedoch die verschiedenen chromotropen Substanzen nicht differenziert zur Darstellung bringt. Die Vermutung, daß polare Lösungsmittel die Bindung zwischen basischem Farbstoff und chromotroper Substanz verändern und daß die Aufhebung der Farbreaktion für verschiedene Substanzen bei verschiedenen charakteristischen Lösungsmittelkonzentrationen erfolgt, wird *in vitro* an Serienverdünnungen mit Toluidinblau geprüft. Die purpurrote Farbe konnte mit polaren Lösungsmitteln ausgelöscht werden. Die zur Auslöschung benötigte Menge des Lösungsmittels ist für verschiedene chromotrope Substanzen charakteristisch und die damit verbundene dielektrische Änderung ist für eine spezifische Substanz von gleichbleibender Größenordnung.

The Thyroxine Sensitivity of Normal and Thyroidectomized Rats

Though several investigators mention that thyroidectomized animals are more sensitive to thyroxine than normal ones, only very few quantitative data are available. Meyer and Wertz[1] administered thyroxine to normal and thyroidectomized rats during three days, measured O_2-consumption on the fifth day, and found that the latter rats are 25–30 times as sensitive as the former. Repeated doses of thyroxine are known to be more effective than the same amount administered in a single dose[2], and therefore these data could not be valid for the relative effectiveness of single doses. The minimal effective dose of thyroxine in thyroidectomized animals has apparently not been established and was therefore investigated.

Methods:—Male rats of approximately 250 g bodyweight and well accustomed to the determination of O_2-consumption were used. O_2-consumption was estimated before and 24 hours after the subcutaneous administration of thyroxine (Schering) in the somewhat modified apparatus of Belák and Illényi[3].

The O_2-consumption of the thyroidectomized animals was 30–40 p. c. below normal before the administration of thyroxine, while that of the controls was within normal limits.

Fig. 1 demonstrates that subcutaneous injection of 2 μg are followed after 24 hours by a marked rise of O_2-

[1] A. E. Meyer and A. Wertz, Endocrinol. *24*, 683 (1939) cit. Ber. Physiol. *114*, 458.

[2] E. Le Breton and G. Schaeffer, C.R. Soc. Biol. *118*, 445 (1935).

[3] K. Belák and A. Illényi, Biochem. Z. *281*, 27 (1935).

consumption in the thyroidectomized rat, while even 1 mg has no effect on the normal animal. Neither was a

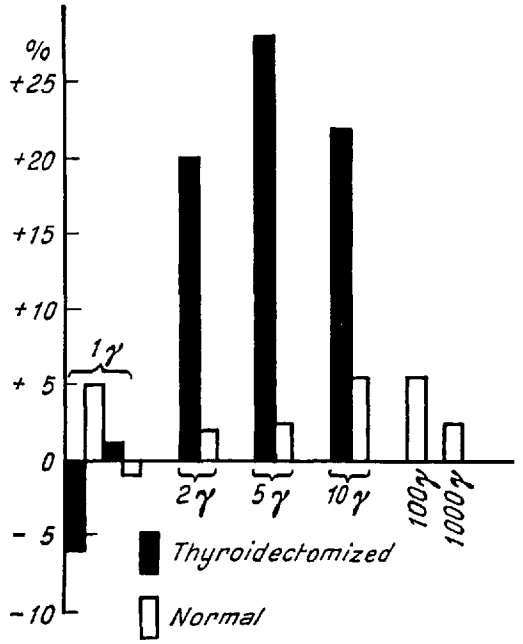

Fig. 1. – Changes in the rate of basal metabolism 24 h after a single dosis of thyroxine. The 0-line represents the B.M. before thyroxine injection.

more delayed action of single doses of thyroxine observed in the normal animals. Daily estimations of O_2-consumption for at least one week were all within the

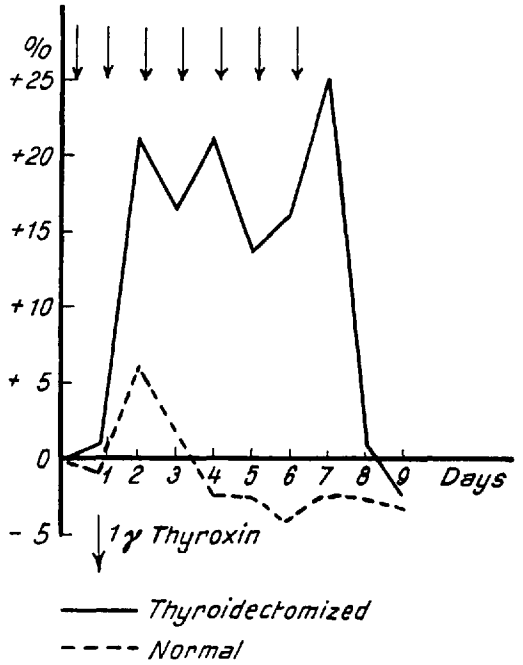

Fig. 2. – Changes in the rate of metabolism produced by 7·1 μg thyroxine. The 0-line represents the B.M. on the first day of the experiment.

normal range. In these experiments—performed during the summer months—single doses of thyroxine proved to be more than 500 times as effective in thyroidectomized animals as in normal rats. The doses of thyroxine employed are considerably smaller than hitherto used.

Fig. 2 and 3 show that repeated doses of thyroxine highly effective in thyroidectomized animals fail to raise the rate of metabolism in the normal animal. Daily doses of 10 μg or more ultimately raise the O_2-consumption of normal rodents also, and under these conditions a similar relative sensitivity to that described by MEYER and WERTZ[1] was found.

Two explanations offer themselves for the great difference found between normal and thyroidectomized animals. The first would be that animals depleted of thyroxine and having therefore a low rate of metabolism (– 30 to – 40%) react to much smaller doses than

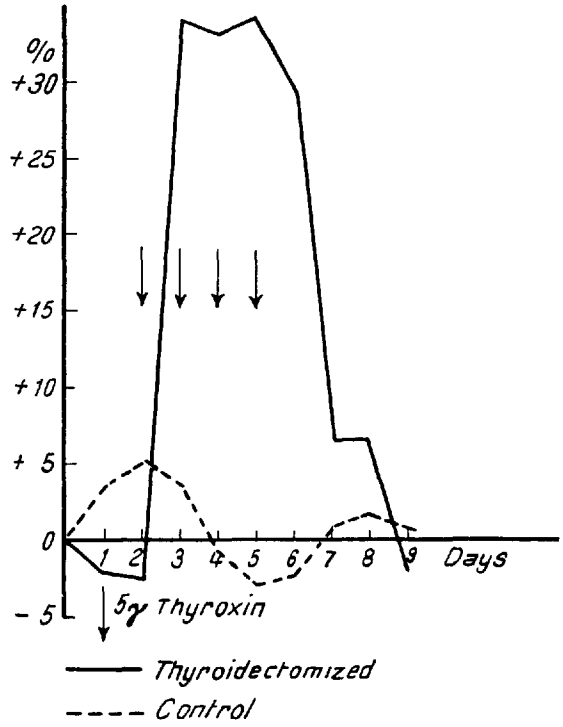

Fig. 3. – Changes in the rate of metabolism produced by 4·5 μg thyroxine. The 0-line represents the B.M. on the first day of the experiment.

animals with plenty of thyroxine in the tissues. The fact that thyroxine doses effective in normal subjects may have hardly any effect in hyperthyroidism could support this assumption. The second obvious explanation would be that the normal thyroid gland is able to buffer the action of large doses of thyroxine. The elective storage of iodine in the thyroid gland may be considered suggestive in this respect. The marked difference between the relative sensitivity towards single and repeated doses is not easy to explain in either case. Experiments devised to investigate this problem are in progress.

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Zusammenfassung

Schilddrüsenlose Ratten mit einer Stoffwechselsenkung von 30–40% reagieren auf die einmalige Injektion von 2 μg Thyroxin Schering nach 24 Stunden mit einer Stoffwechselsteigerung von etwa 20%, während der

[1] A.E. MEYER and A. WERTZ, Endocrinol. *24*, 683 (1939), cit. Ber. Physiol. *114*, 458.

Stoffwechsel normaler Ratten selbst von der einmaligen Injektion von 1 mg unbeeinflußt bleibt. Schilddrüsenlose Tiere sind daher einmaligen Thyroxingaben gegenüber mehr als 500mal so empfindlich als normale. Prüft man die Wirkung wiederholter Gaben, so ist der Unterschied bedeutend geringer und den Angaben von MEYER und WERTZ etwa entsprechend.

Substances synaptolytiques, curarisantes et nicotinolytiques

On sait que différentes substances, parmi lesquelles la nicotine, le di-isopropylfluorophosphonate (DFP)[1], le hexa-éthyltétraphosphonate (HETP)[2] et l'acétylcholine, excitent les synapses ganglionnaires végétatifs et peuvent les paralyser ensuite.

D'autres substances paralysent les synapses autonomes périphériques, sans les stimuler d'abord. Parmi ces substances signalons le tétra-éthylammonium (TEA)[3], le diéthylaminoéthyl-ester de l'acide phénylcyclopentane carboxylique (Parpanit)[4], la phénothiazinyl-éthyl-diéthylamine (2987 R. P. ou Diparcol)[5], la tubocurarine, la méthyltubocurarine[6], le diiodoéthylate de bis(quinoléyl-8')-1,5-pentane (3381 R. P.)[7] et le lauryl-diméthyl-aminoéthanol (764 L.)[8]. Le Parpanit et le Diparcol paralysent également les éléments périphériques de l'innervation post-synaptique du parasympathique (action atropinique), et dépriment les centres nerveux moteurs (action anticonvulsive)[9]. Parmi ces substances synaptolytiques, certaines (tubocurarine, méthyltubocurarine, 3381 R. P. et 764 L.) sont également curarisantes, alors que les autres ne le sont pas.

Signalons, d'autre part, une substance synthétique, le triiodure de tri(triéthylammoniumméthoxy)-1,2,3- benzène (2559 F.), qui est très curarisant, tout en n'étant pas synaptolytique[10]. Les propriétés curarisantes et synaptolytiques ne sont donc pas liées l'une à l'autre.

Remarquons également que ces substances synaptolytiques et curarisantes sont dépourvues de propriétés anticholinestérasiques, à l'exception du Diparcol qui inactive, d'une manière sélective, la «pseudo-cholinestérase» *in vitro* et *in vivo*, sans inhiber la «vraie cholinestérase»[1]. Ces faits montrent que l'action curarisante et synaptolytique est indépendante de l'action anticholinestérasique.

Parmi les substances synaptolytiques et curarisantes, certaines possèdent une action antagoniste très prononcée vis-à-vis de la nicotine et les substances nicotiniques.

Nous avons observé que le Parpanit[2] et le Diparcol[3] protègent l'organisme vis-à-vis de très fortes doses de nicotine et de substances nicotiniques (acétylcholine, autres dérivés cholinergiques de la choline, DFP, HETP, hordénine, pilocarpine). Ces composés nicotinolytiques empêchent la bradycardie, les irrégularités cardiaques, la fibrillation cardiaque, l'hypertension, le bronchospasme, l'hyperpéristaltisme, la salivation, le myosis, les convulsions et les trémulations musculaires, provoqués par la nicotine et les substances nicotiniques, particulièrement le DFP. Le Parpanit et le Diparcol protègent également, mais à un moindre degré, le centre respiratoire contre l'action paralysante des substances nicotiniques.

Les curarisants synaptolytiques (tubocurarine, méthyltubocurarine, 3381 et R. P. 764 L.) possèdent les mêmes propriétés que le Parpanit et le Diparcol, mais ne protègent pas contre les actions muscariniques que certaines substances nicotiniques (acétylcholine et dérivés de la choline) possèdent également.

Le tétra-éthylammonium, substance synaptolytique très active, mais non curarisante, protège contre la bradycardie, le bronchospasme, l'hyperpéristaltisme, la salivation, l'hypertension et le myosis nicotiniques, mais ne protège pas contre les convulsions et les trémulations musculaires nicotiniques. Le 2559 F., curarisant non synaptolytique, protège contre les convulsions et les trémulations musculaires, mais non contre les autres actions nicotiniques.

Ajoutons que l'atropine protège contre la bradycardie, le bronchospasme, l'hyperpéristaltisme, la salivation et le myosis nicotiniques, mais non contre l'hypertension artérielle, les convulsions et les trémulations musculaires nicotiniques.

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Summary

Diethylaminoethyl ester of phenyl-cyclopentane carboxylic acid (Parpanit) and diaminoethylphenothiazine (2987 R. P., Diparcol), substances paralysing the autonomic synapses, the postsynaptic cholinergic innervation and the motor centers, protect very actively against nicotinic and cholinergic intoxication. Parpanit has no anticholinesterase action, while Diparcol inactivates selectively pseudo-cholinesterase *in vitro* and *in vivo*.

Curarizing and synaptolytic agents as tubocurarine, methyltubocurarine, diiodoethylate of bis (quinoleyl-8'-1,5-pentane) (3381 R. P.) and lauryl-dimethyl-aminoethanol (764 L.), protect against nicotinic intoxication,

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