compound fractions were expressed in mg% of SH-glutathione. Thiocyanate level in the serum of experimental animals was estimated according to the method of ALDRIDGE[3].

Results. A significant increase in the relative thyroid weight and thiocyanate level in serum was found in the group of rats fed on cabbage in comparison with the controls (Table). This finding was taken as evidence of the goitrogenic activity of the cabbage. The total sulfhydryl compounds and protein-bound sulfhydryl groups in the liver are significantly higher in the group of rats fed on cabbage than in the controls. The values of non-protein sulfhydryl compounds determined by means of polarography and SH-glutathione level determined by means of glyoxalase in deproteinized liver homogenates and in deproteinized blood are practically equal in both groups of rats, with no significant differences (Table). From these results it can be concluded that the effect of goitrogenic cabbage induces a striking increase of protein-bound sulfhydryl groups and thereby of total sulfhydryl compounds in the liver of rats receiving goitrogenic cabbage. Meanwhile, the SH-glutathione level in the liver and blood of both the experimental groups of rats remains stable.

Discussion. Goitrogens, on entering the organism, cause a decrease in the production of thyroid hormone, bringing about a hypothyroidal condition in the whole organism, a lowered overall metabolic rate and lowered oxygen utilization by the tissues. This inhibitory influence of goitrogens may be a causative factor in the increased level of protein-bound sulfhydryl groups and thus in that of the total sulfhydryl compounds in the liver of rats receiving goitrogenous cabbage. The results obtained in this experiment are not sufficient to provide a satisfactory explanation for such an enormous increase in the total and protein sulfhydryl compounds in rat liver, following feeding with goitrogenous cabbage. As against this view, we presume that in this particular case a shift of the oxidation-reduction equilibrium of sulfhydryls and disulfides takes place on the surface of the protein enzymes, thus affecting the activity of several enzymes.

On the assumption that cabbage goitrogens and thiouracil act through the same mechanism as goitrogenic substances, our results are in contradiction with those of HOUSSAY et al.[4] and of CAPRA[5], who found a marked increase in SH-glutathione values in the liver, kidneys and blood of rats receiving thiouracil or methyl-thiouracil. On the other hand, our results are in good agreement with the findings of VIRTANEN[6], who failed to observe changes in SH-glutathione level in deproteinized homogenate of rat livers, following thiocyanate in doses inducing goitrogenic effect. HOPSU et al.[7] likewise found an increased level of protein-bound sulfhydryl groups in the thyroid of guinea-pigs following methyl-thiouracil.

Zusammenfassung. Eine statistisch signifikante Erhöhung der eiweissgebundenen SH-Stoffe, somit auch der totalen SH-Stoffe in der Leber mit strumigenem Kohl gefütterter Ratten, wurde festgestellt. Im Gehalt nicht eiweissbundener SH-Stoffe (SH-glutathion) in Leber und Blut nach Kohlverfütterung kam es zu keiner Veränderung.

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[3] W. N. ALDRIDGE, Analyst *70*, 474 (1945).
[4] B. A. HOUSSAY, C. MARTINEZ, and R. CAPUTTO, Rev. Soc. Argentina Biol. *23*, 248 (1947).
[5] P. CAPRA, Boll. Soc. Med. Chim., Pavia, *61*, 7 (1947).
[6] A. J. VIRTANEN, Exper. *17*, 241 (1961).
[7] V. K. HOPSU, K. J. HEIKKILÄ, and M. HÄRKÖNEN, Acta Endocr. (Kbh.) *34*, 605 (1960).

Storage Function and Amine Levels of the Adrenal Medullary Granules at Various Intervals after Reserpine Treatment

It has previously[1] been found that the amine granules of the adrenal medulla take up and concentrate catecholamines and 5-HT *in vitro* at low external concentrations of the added amines. The storage mechanism is activated by ATP and Mg^{++} and is blocked by reserpine at low concentrations.

In the present investigation adrenal, medullary granules were examined in this way at different intervals following injection of a single dose of reserpine (5 mg/kg) to rabbits.

Methods. Rabbits weighing about 2.5 kg were injected with reserpine (5 mg/kg) intravenously. At different intervals following injection (3–72 h) the rabbits were killed by an intravenous injection of air. The adrenals were immediately removed and chilled with ice. The procedure described below is essentially the same as that of CARLSSON, HILLARP, and WALDECK[1]. The medulla with some adhering cortical tissue was rapidly dissected and homogenized with a loose-fitting plastic pestle for about 20 sec in 7 ml of 0.3 *M* sucrose. To remove unbroken tissues and cells, but at the same time to prevent loss of amine granules, the homogenate was centrifuged at $800 \times g$ for 5 min. The supernatant was centrifuged at $20{,}000 \times g$ for 20 min. The sediment was suspended in 0.5 ml 0.3 *M* sucrose. The granule suspension was transferred to 1.0 ml of an incubation mixture (at 0°) containing glycylglycine (0.31 *M*), unlabelled adrenaline or noradrenaline (25 µg/ml), C^{14}-labelled adrenaline or noradrenaline (4.3–5.6 µg/ml), $MgCl_2$ (0.0025 *M*) and ATP (0.0025 *M*).

Incubation was performed without shaking at 31° for 30 min, after which the suspension was chilled to 0°, diluted 30 times with cold 0.5 *M* sucrose and – after about 1 h at 0° – centrifuged at $74{,}000 \times g$ for 30 min. After thorough rinsing of the tubes, the granule sediment was extracted with 5.0 ml of 0.01 *N* HCl in 98% ethyl alcohol. The catecholamine content of the extracts was determined spectrophotofluorimetrically[2]. The C^{14}-amine content was determined directly in a liquid scintillation counter. The identity of the C^{14}-compounds has previously been checked by paper chromatography[1].

Pure nucleotides from the Pabst laboratories and pure reserpine generously supplied by Ciba Ltd (Basel) were used. DL-Adrenaline-methyl-C^{14} (21.9 mC/m*M*) and DL-noradrenaline-7-C^{14} were purchased from Commissariat à

[1] A. CARLSSON, N.-Å. HILLARP, and B. WALDECK, Med. exp. *6*, 47 (1962).
[2] Å. BERTLER, A. CARLSSON, and E. ROSENGREN, Acta physiol. scand. *44*, 273 (1958).

l'Énergie Atomique, France, and stock solutions in 0.01 *N* HCl were stored at −30°.

Results and Discussion. In each experiment one reserpine-treated and one control animal were used. In each experiment the amount of incorporated C^{14}-amines after reserpine injection is given in % of the control value, and amine levels are given in µg per pair of adrenals.

Reserpine caused a pronounced blockade of the uptake of adrenaline or noradrenaline by the storage granules (Figure). The effect lasted 12 to 24 h. There was no detectable difference between results obtained with labelled adrenaline and noradrenaline. After about 48 h the incorporation was restored while the level of catecholamines was still very low in the adrenal medulla.

It is known that after a single injection of a large dose of reserpine (2–5 mg/kg intravenously) to rabbits, the pharmacological effects (sedation, miosis, ptosis) disappear within the first two days whereas the concentrations of tissue catecholamines still remain low. The return to normal values of catecholamines requires about 2 weeks[3].

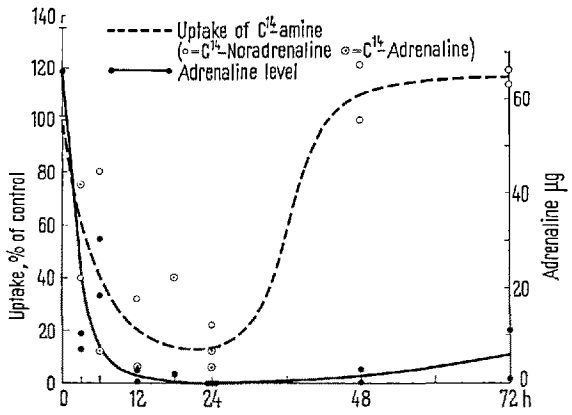

Fig. 1. Adrenaline level of adrenal medulla and uptake of C^{14}-catecholamines by adrenal medullary granules *in vitro* at various intervals following injection of reserpine (5 mg/kg i.v.) to rabbits.

Thus there is a much better correlation between the pharmacological effects of reserpine and storage function than between these effects and amine levels, which indicates that the actual content of amines of the tissues is of only minor importance for the function.

HILLARP[4] has shown that there are different fractions of amines in the adrenal medulla. It may be that the smaller labile fraction will be restored rather soon after the depletion achieved by reserpine and that only this fraction is necessary for function, while the larger stable fraction may still be completely depleted of amines[5].

Zusammenfassung. Der Speichermechanismus der Amingranula des Nebennierenmarkes von Kaninchen wurde in verschiedenen Intervallen nach Injektion einer einzigen Dosis Reserpin (5 mg/kg intravenös) untersucht.

Der Speichermechanismus war 12 bis 24 h nach der Injektion blockiert, aber nach 48 h wieder normal, während der Catecholamingehalt der Amingranula immer noch vollständig depletiert war.

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[3] A. CARLSSON, E. ROSENGREN, Å. BERTLER, and J. NILSSON, *Psychotropic Drugs* (S. GARATTINI and V. GHETTI, Eds., Elsevier Publishing Company, Amsterdam 1957), p. 363.

[4] N.-Å. HILLARP, Acta physiol. scand. *50*, 8 (1960).

[5] *Acknowledgments.* This work has been supported by a grant (-2854) from the United States Public Health Service. – I wish to express my thanks to Mr. B. WALDECK for the C^{14}-determinations and other technical assistance.

An *in vivo* Bioassay for TSH-Releasing Factor (TRF)

When it was proposed in this laboratory to engage in a program studying the hypothalamic control of the secretion of TSH, a bioassay for the postulated TRF (TSH-releasing factor) had to be chosen. Since none was available in the literature which would adequately meet our criteria, it was decided that a new method should be set up which would fulfill the various requirements outlined below.

(1) It was proposed to accept as a working hypothesis that thyroxine inhibits the secretion of endogenous TSH at least in part through a negative feedback mechanism acting directly at the level of the adenohypophysis. (2) The method should be reasonably sensitive for TSH. (3) The method should be simple enough to allow in a day's work the testing of at least 10 unknowns (fractions of the effluent of some separation method). (4) It should utilize an animal in which hypophysectomy is a simple procedure so that the various fractions could be tested for direct (TSH) activity or transhypophysial (TRF) activity. (5) The method should allow the testing of crude extracts —which precluded considering an *in vitro* assay[1] for routine studies.

On the basis of these premises it was decided to use a modification of the method of MCKENZIE[2]. Because of the difficulty of routine performance of hypophysectomy in mice (the animal species used in the original method of MCKENZIE), it was decided to use the rat. In keeping with the concept that thyroxine inhibits TSH release at least in part by acting at the pituitary level, it was proposed to maintain the animals under a low load of thyroxine (rather than without thyroxine at all), so as to increase their (peripheral) sensitivity to TSH while at the same time retaining the ability of the pituitary to respond to TRF be secreting TSH.

The proposed bioassay for TRF-activity is as follows:

Rats, males, body weight 50 g when received in the laboratory, are kept in a constant temperature room (22°C ± 2), on a low iodine diet (Nutritional Biochemicals Co., Cleveland, USA) and double (glass) distilled water as drinking fluid for 10 days. They are given one i.p. injection of 3.0 µc I^{131}, carrier free, followed 5 h later by one s.c. injection of 5 µg *l*-thyroxine. They are used in the test 65–70 h after injection of *l*-thyroxine: under

[1] R. GUILLEMIN and A. V. SCHALLY, Endocrinology *65*, 555 (1959).

[2] J. M. MCKENZIE, Endocrinology *63*, 372 (1958).