that the Golgi bodies lose their form, leaving the centrosome free. The movement and subsequent division is enhanced due to irradiation. It has also been suggested by LINDERGREN et al.[11] that the centriole is a primary radiation target. Since the centrosome encloses the centrioles, and if the latter are radiation target, the movement and division of centrosome could easily be explained. As stated earlier, due to the small size of this body, no positive explanation could be offered[12].

Zusammenfassung. Röntgenstrahlen haben einen bemerkenswerten Einfluss auf die Inklusionen der männlichen Geschlechtszellen, wie er durch die Verklumpung des Chromatins, die Aggregation der Mitochondrien und die Granulanatur des Golgikörpers evident wird.

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Department of Zoology, University of Rajasthan, Jaipur (India), April 22, 1963.

[11] C. C. LINDERGREN, D. D. PITTMAN, and A. YUASA, Trans. N.Y. Acad. Sci. *21*, 524 (1959).

[12] We wish to thank Prof. L. S. RAMASWAMI for various facilities in the Department. Our thanks are also due to Dr. D. G. OJHA and Dr. K. N. PANJAL for the irradiation facilities in the M.G. Hospital, Jodhpur.

Changes in Protein-bound Sulfhydryl Group Concentration in the Liver of Rats Fed Cabbage

The effect of goitrogenic substances on tissues in which the oxygen utilization becomes reduced is well known, and it may be expected that the goitrogenic agents will affect the level of sulfhydryl compounds in various organs of experimental animals. We have found a marked increase in the total sulfhydryl compounds in the liver of rats after feeding with goitrogenous cabbage[1]. These results were an incitement to a further study of the problem.

Material and Methods. In the present experiment 20 white female rats of the Wistar strain were fed winter cabbage (*Brassica oleracea* var. capitata) for 180 days. In addition, they received Larsen's diet, but no water. (Average daily consumption per rat was about 35 g of cabbage and 7 g of Larsen.) The controls, a group of 10 rats, had Larsen's diet and water *ad libitum*. At the end of the experiment, the rats were sacrificed under ether anaesthesia by a puncture of the ventral aorta, and the relative weight of the thyroid and of the liver was determined. The liver was weighed immediately on being taken out and total sulfhydryl compounds and non-protein sulfhydryl compounds were determined by polarographic estimation. The difference between total sulfhydryl compounds and non-protein sulfhydryl compounds represented the value of protein-bound sulfhydryl groups. 2% sulfo-salicylic acid were used for the deproteinization of liver homogenates. In addition, SH-glutathione was determined in the deproteinized liver homogenates and in hemolysed and deproteinized blood by the manometric method with the aid of glyoxalase, prepared from baker's yeast. The enzymatic estimation of SH-glutathione was done according to WOODWARD's method[2]. All sulfhydryl

[1] J. SEDLÁK, Nature *192*, 377 (1961).

[2] G. F. WOODWARD and E. G. FRY, J. biol. Chem. *97*, 465 (1932).

Determinations performed in the experiment

Type of estimation	Experimental group E (n = 20)[a]	Controls C (n = 10)	Significance of difference
Thyroid weight in mg/100 g body weight	9.01 ± 1.77[b]	6.76 ± 0.49	E > C significant $p \lessapprox 0.001$
Liver weight in g/100 g body weight	3.71 ± 0.31	3.44 ± 0.34	E > C non-significant
Thiocyanate in serum according to Aldridge, in mgm% SCN^{-1}	1.08 ± 0.19	0.36 ± 0.10	E > C significant $p \lessapprox 0.001$
Total sulfhydryl compounds in liver estimated polarographically	520.60 ± 155.50[c]	372.30 ± 68.30	E > C significant $p \lessapprox 0.02$
Protein bound sulfhydryl groups in liver	337.6 ± 123.90	193.80 ± 68.70	E > C significant $p \lessapprox 0.01$
Non-protein sulfhydryl compounds in liver estimated polarographically	187.70 ± 27.50	176.20 ± 19.10	E > C non-significant
SH-glutathione in liver estimated enzymatically	188.20 ± 43.30	195.40 ± 38.60	C > E non-significant
SH-glutathione in blood estimated enzymatically	40.96 ± 7.34	43.40 ± 8.46	C > E non-significant

[a] n = Number of rats in the experiment. [b] $(\bar{X} + \sigma)$ = Average + standard deviation. [c] All sulfhydryl compound fractions are expressed in mg% of SH-glutathione in fresh liver or blood.

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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Endocrinological Institute-SAV, Bratislava (Czechoslovakia), June 7, 1963.

[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).