Die vorliegenden Ergebnisse stimmen mit denjenigen von ERSHOFF[4] bezüglich der Toxizität der Verbindung Schilddrüse/Reserpin überein und bestätigen, dass diese Wirkung durch verstärkte Herzschädigung infolge einer grösseren Verringerung der Adenosinnucleotide des Herzgewebes eintritt.

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Riassunto

L'aggiunta di reserpina ad una dieta contenente estratto di tiroide, produce nei ratti un aggravamento della sintomatologia tireotossicosa caratterizzato da aumento della mortalità, maggiore inibizione dello sviluppo corporeo ed ulteriore caduta delle concentrazioni cardiache di ATP, ADP e AMP.

Si suggerisce che tale effetto sia dovuto ad un potenziamento da parte della reserpina dell'azione della tiroide sul metabolismo del tessuto cardiaco.

Studies on the Hypoglycemic Effect of 'Vincamin'

In the past years several authors studied the effect of Rauwolfia alkaloids on the blood sugar level, but the results were contradictory. Some reports demonstrated increase of blood sugar level, others observed definite decrease[1–4]. An alkaloid 'Vincamin', isolated from *Vinca minor*[5–9] in hypotensive effect similar to Rauwolfia alkaloids decreases the blood sugar of rabbits, as observed HANO[10].

Besides studying the hypotensive effect of Vincamin[11], alterations of blood sugar values due to the drug were examined, and the mechanism of this action has also been studied.

Method. In preliminary experiments, it has been established in guinea pigs, rabbits, and rats that the alterations of blood sugar are especially definite in the latter. Our studies were made in Wistar rats of both sexes weighing 200–300 g. The animals had been starved for 24 h, blood samples were taken from the tails, blood sugar determinations were made with the method of Hagedorn-Jensen. Vincamin was administered intraperitoneally, adrenaline subcutaneously, dextrose through a gastric tube using 40% solution.

Our data (see Table) indicate that 0.2 mg/kg Vincamin causes definite fall in blood sugar values. The decrease can be observed in all animals during the first 30 min – 22 mg% decrease as average – and the effect disappears only after 4 h.

After administration of 4 g/kg dextrose, blood sugar values increase – 28 mg% as average after 30 min. If 0.2 mg/kg Vincamin has been given simultaneously with dextrose, no elevation of blood sugar level was observed.

The administration of adrenaline (25 γ/kg) causes definite hyperglycemia. This effect is not abolished with Vincamin (0.2 mg/kg).

Discussion. Our results indicate that blood sugar values of rats decrease after administration of Vincamin in acute experiments. It is generally accepted that hyperglycemia provoked by the administration of dextrose and adrenaline do not develop by the same mechanism[12]. Hyperglycemia after giving dextrose is caused by the absorbed quantity of sugar, while in the latter case it comes about by hepatic glycogenolysis.

Vincamin, as demonstrated, abolishes hyperglycemia caused by the administration of dextrose. The increase of blood sugar after administration of adrenaline remains unchanged. These observations indicate, that hypoglycemia caused by Vincamin is not due to inhibition of hepatic glycogenolysis. This phenomenon should probably be considered as the central effect of Vincamin. The intermediary effect of insuline is under study.

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Changes of Blood Sugar Values after Administration of Vincamin, Dextrose + Vincamin, resp. Adrenaline + Vincamin

	No. of animals	Dosage	Fasting values mg%	Blood sugar values in mg% after 30 min	60 min	120 min	240 min
Vincamin	12	0.2 mg/kg i. p.	114 (100–130)	92 (84–105)	94 (84–110)	94 (84–110)	114 (104–132)
Dextrose	5	4 g/kg p. os	94 (92–97)	125 (110–148)	131 (121–134)	133 (126–136)	—
Dextrose + Vincamin	5	4 g/kg 0.2 mg/kg i. p.	116 (98–123)	108 (86–121)	112 (98–125)	102 (87–119)	—
Adrenaline + Vincamin	5	25 /kg 0.2 mg/kg i. p.	100 (66–124)	136 (123–155)	163 (127–210)	162 (155–168)	—

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Zusammenfassung

Nach Verabreichung von Vincamin sinkt bei akuten Versuchen an Ratten der Blutzuckerspiegel. Die nach Dextroseverfütterung verursachte Hyperglykämie wird durch Vincamin verhindert, der Blutzuckeranstieg jedoch bleibt nach Adrenalin unbeeinflusst.

Folic Acid Degradation by the Peroxidase from *Cicer arietinum*

In contrast to most other members of the vitamin B-complex, folic acid content of seeds has been found to decrease during germination[1,2].

Investigations on *Cicer arietinum*, designed to explore the mechanisms by which folic acid may be inactivated in seeds, have demonstrated the presence of a peroxidase which converts folic acid to 2-amino-4-hydroxy-6-formyl-pteridine and *p*-amino-benzoyl-glutamic acid.

Methods. Seeds of *C. arietinum* were sown on sterile cotton soaked in water and were allowed to germinate in the dark for 96 h.

Enzyme extracts. 15 g of seedling were extracted with 100 ml of 0.1 *M* phosphate buffer pH 5.5. After 1 h the extract was centrifuged for 10 min at $10{,}000 \times g$ and the supernatant thus obtained was employed.

Heated extract. 20% crude extract prepared from seeds germinated for 96 h was heated at 90°C for 20 min, the coagulated proteins were removed by centrifugation and the supernatant was the source of active extract.

Enzyme assay. Activity was measured by estimating the diazotisable amine liberated from folic acid by the BRATTON and MARSHALL test[3] as described previously[4].

Results. Crude enzyme extracts prepared from *C. arietinum* have been found actively to liberate the aromatic amine from folic acid. The activity developed progressively during germination. In 96 h of germination in the dark, the seedling extracts could break down 50% of the added folic acid (300 µg) in 60 min. Dry seeds were inactive.

Attempts to study the cofactor requirements showed that extensive dialysis against water was not adequate to remove the cofactors. Resort to versene dialysis, followed by removal of versene by further dialysis against water, proved effective. Such preparations could be activated by the addition of heated extract. These observations indicate that metal ions may be required for the optimum activity of the enzyme system. In presence of heated extract, activity could be observed only in presence of air. The system was inactive under anaerobic conditions. Heated extracts prepared as described in the section on methods could be inactivated by further heating and reactivated by the addition of H_2O_2. As previous observations have demonstrated that folic acid may be inactivated by the peroxidatic action of methemoglobin[5], experiments were carried out to determine if a similar mechanism was involved in the present system. The results showed that in presence of enzyme extracts containing 100 µg of protein, folic acid could be degraded only if the inactivated heated extract was enriched with H_2O_2. The system was not active in absence of air. In presence of higher concentrations of enzyme (700 µg), folic acid degradation could be demonstrated with H_2O_2 alone without the addition of the heated extract. It was also observed that systems in which the heated extract could be dispensed with, were active both under aerobic and anaerobic conditions. In presence of oxygen, the extent of

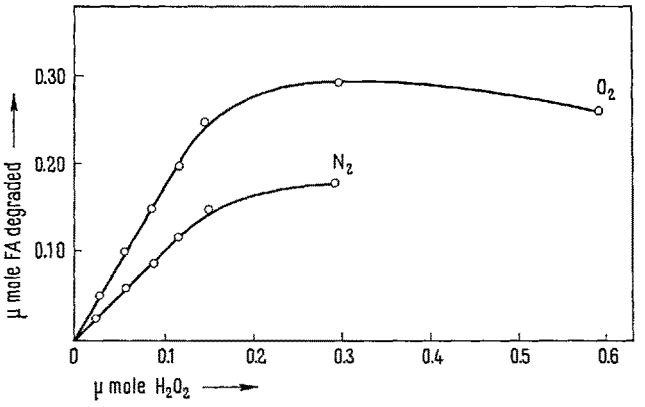

Fig. 1. Effect of varying concentrations of H_2O_2 on folic acid degradation by enzyme extract.
Reaction mixture consisted of FA 300 µg, PO_9^{-}. Buffer 0.025 M. pH 5.5, V. W. E. (700 µg protein) and varying amounts of H_2O_2. Total volume = 3.0 ml. Incubation time = 60 min. Temp. = 37°C.

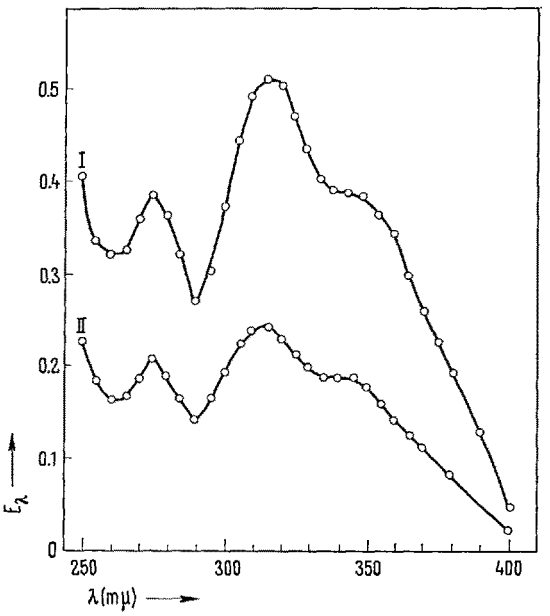

Fig. 2. Identification of carbonyl derivative as 2-amino-4-hydroxy-6-formyl pteridine.
Curve I. Acetic acid eluate containing enzymic degradation products of folic acid. *Curve II.* 2-amino-4-hydroxy-6-formyl pteridine in acetic acid.

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