75% of the bound sialic acid of serum. By analogy, therefore, it may be presumed that the combination of WBH with red blood cells involves the same or similar site on the surface of the cell which serves as a substrate for the viral enzyme or RDE.

There are two further observations of a negative nature. First, WBH could not be eluted from the erythrocytes once agglutination had occurred, and secondly, WBH was unable to cleave sialic acid from the various mucoproteins shown in Table I or from sialyllactose. Of the 3 successive steps involved in viral agglutination-adsorption leading to agglutination, enzymic destruction of receptor site, and elution of virus—the enzymic and elution stages can therefore be clearly excluded in the case of WBH. Thus the mode of action of WBH most closely resembles that of the heat-inactivated or "indicator" virus which retains the capacity to agglutinate red cells, an effect which can be inhibited by mucoproteins, but which can no longer be eluted due to the loss of its enzymic function.

Summary. Mucoproteins known to be inhibitors of viral agglutination inhibited agglutination of rabbit erythrocytes by the wax bean hemagglutinin (WBH). Pretreatment of red blood cells with influenza (PR 8) or Newcastle Disease virus reduced susceptibility of such cells to agglutination. The enzymatic release of sialic acid from rabbit serum by a receptor-destroying enzyme prepared from *Cl. perfringens* was accompanied by a loss in inhibitory activity of serum against WBH agglutination. These and related observations suggest that the site of reaction of WBH with erythrocytes is similar, if not identical, to that of the viral receptor site on the surface of the cell.

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A Mechanism of Action for Antithyroid Activity of Reserpine.* (24540)

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Since the introduction of reserpine as a therapeutic agent, reports have appeared ascribing an antithyroid activity to this substance. Chronic reserpine therapy reportedly resulted in "normalization" of the basal metabolic rate in 15 hyperthyroid patients(1), and in peripheral inhibition of thyroxin by reserpine(2,3), as well as a more direct action by reserpine upon the thyroid(4,5). Determination of basal metabolic rate or degree of iodine

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TABLE I. Comparative Drug Effects upon Dehydrogenase Enzyme Activity of Thyroid.

Treatment	No. of animals	Avg wt of dried thyroids, mg	μg of dye reduced/mg dried tissue, mean ± S.E.	P
(a) Normal controls	28	2.1	14.2 ± .6	
(b) Reserpine	30	2.5	10.9 ± .5	*vs* (a) <.0001
(c) Synthroid Na	15	2.5	7.9 ± .7	*Idem*
(d) Thiouracil	15	5.4	30.9 ± .7	"
(e) Thyrotropar	14	2.0	20.3 ± 1.2	"
(f) Reserpine + thiouracil	10	4.1	29.6 ± 2.1	*vs* (b) $<2 \times 10^{-12}$
(g) " + Thyrotropar	20	1.6	19.1 ± .8	*Idem*

uptake was employed in the above reports for measuring any effect by reserpine upon the thyroid gland. In the present investigation the effect of reserpine upon endogenous dehydrogenase enzyme activity of the thyroid was quantitated. These results were compared to results obtained with substances whose mechanism of action upon the thyroid is known.

Methods. Male and female adult rats of our inbred colony of the Denver Strain were used. No sex difference was found in control or treated animals. Endogenous dehydrogenase enzyme activity was quantitated employing the tetrazolium technic(6) as modified for tissue slices(7). Triphenyltetrazolium chloride was used as indicator in a modified Kreb's solution(8). Incubation was carried out aerobically with shaking at 37°C for 2 hours. After killing the animal by a sharp blow on back of the head, both lobes of the thyroid were immediately dissected free and incubated without further sectioning. The amount of dye reduced by both lobes was determined colorimetrically after extraction with acetone. This value was converted to μg of dye reduced/mg of air dried tissue. Pure powdered reserpine‡ was dissolved in a minimum of glacial acetic acid and brought to the desired volume with 10% propylene glycol. The previously reported dosage schedule of 100 μg/kg/day administered subcutaneously for 14 days was employed(9). Thiouracil was given in the drinking water as a 0.1% solution for 10 days(10). Thyrotropin, as Armour's Thyrotropar, was administered intramuscularly at a dose of 2 units/rat in 4 divided doses over a 24 hour period(11). Synthroid Na, the synthetic l-thyroxin preparation of Travenol Labs, was suspended in normal saline and injected subcutaneously daily for 8 days at 50-75 μg/rat/day. To observe effects of combined reserpine-thiouracil administration, thiouracil was added to drinking water 4 days after initiation of daily reserpine treatment, and both substances given together for another 10 days. Thyrotropin, when administered together with reserpine, was given on the last day of the regular 14-day reserpine schedule.

‡ Powdered reserpine (Serpasil) was obtained through courtesy of Dr. F. F. Yonkman, Ciba Pharmaceutical Products.

Results. Mean values of enzyme activity obtained with each experiment are given in Table I. P values were calculated according to conventional methods(12).

Reserpine with the dosage and time schedule employed significantly lowered endogenous dehydrogenase enzyme activity of the thyroid. It has been reported that prolonged administration of reserpine in rats produces slight thyroid enlargement, indicating histologically mild functional inhibition(13). Under our conditions, no histological or macroscopic changes from the normal were seen.

Administration of synthetic l-thyroxin also reduced enzyme activity. Histological examination revealed a mildly resting gland. Thiouracil and thyrotropin significantly raised enzyme activity. With thiouracil administration the gland was typically enlarged and highly vascular. Histological examination of these glands revealed active, hyperplastic thyroid tissue. Thyrotropin, as administered, produced no glandular enlargement or hyperplasia. Microscopically an active epithelial structure was present. Concomitant adminis-

tration of reserpine with either thiouracil or thyrotropin did not alter the effects of these substances upon either enzyme activity or histological findings.

Reduction by exogenous thyroxin of thyroid dehydrogenase enzyme activity was probably due to decreased output of thyrotropin by the pituitary, since administration of exogenous thyrotropin and stimulation of endogenous thyrotropin secretion by thiouracil administration caused an increase in enzyme activity. It thus appears that dehydrogenase enzyme activity of the thyroid reflects degree of thyrotropin activity upon the thyroid.

The reduced enzyme activity, with reserpine administration, does not appear to be due to a direct action upon the thyroid. Administration of thyrotropin, following 13 days of reserpine injection, resulted in a significant increase in enzyme activity over that observed with reserpine alone. In addition, reserpine did not reduce the high levels of enzyme activity due to thiouracil administration. These observations are interpreted as showing that reserpine does not block the action of thyrotropin within the thyroid. It would appear that dehydrogenase enzyme activity of the thyroid is related to the action of thyrotropin on cellular elements of the gland, without regard to thyroxin production by the gland. The high degree of cellular hyperplasia and known reduction of thyroxin secretion in thiouracil treated animals substantiate this belief.

Our data indicate that reserpine has a significant effect upon endogenous dehydrogenase enzyme activity of the thyroid. Due to reserpine's known effects upon higher centers in the central nervous system(13), and the presently suggested relation to thyrotropin activity, it is proposed that the mechanism of action for this reduction by reserpine is *via* the pituitary, to produce a decreased thyrotropin elaboration or release.

This effect of reserpine upon the thyroid *via* the pituitary, although significant, does not appear to be powerful in nature. It did not inhibit the rise of enzyme activity when given with thiouracil. However, some consideration should be given to it when long term reserpine therapy is contemplated. This might be especially true in view of the report that chronic reserpine administration in euthyroid guinea pigs reduced the basal metabolic rate to that of hypothyroid levels(3).

Summary. 1) Endogenous dehydrogenase enzyme activity of the thyroid was quantitated employing the tetrazolium technic. Reserpine and thyroxin significantly lowered enzyme activity, whereas thyrotropin and thiouracil significantly raised it. Thyrotropin, either exogenous or endogenous in origin, prevented reduction of enzyme activity achieved by administration of reserpine alone. 2) The suggestion that these effects are a reflection of thyrotropin activity upon the thyroid is discussed. It is proposed that reserpine does have an antithyroid action and that this action is the result of its effect upon the elaboration or release of thyrotropin by the pituitary.

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