50 μg of isatin-monothiosemicarbazone to produce significant plaque-free areas. Smaller zones were observed with as little as 20 μg but none were seen when 10 μg was used. In no case were these zones equal in size to those produced by just 1 μg of antibiotic W-112; even though paper chromatograms of the synthetic compound were applied to the overlay cultures as soon after virus infection as possible.

Discussion. By adaptation of the Dulbecco plaque technic it is now possible to detect, assay and chromatograph antiviral antibiotics in a manner similar to antibacterial antibiotics. The same method is equally useful in chromatography of synthetic compounds such as isatin-monothiosemicarbazone. This synthetic antiviral agent was chosen for study because it is an agent unquestionably active both in virus infected animals and tissue cultures. Since this compound only inhibits plaque formation of vaccinia virus and not of W. Nile, herpes simplex and Newcastle viruses, this indicates that plaque-free zones are in this case a result of specific influences on the course of virus infection and not due to a subtle, general toxic effect on chick embryo cells.

Summary. A procedure has been developed whereby paper chromatograms of antiviral agents can be developed by applying them to virus infected, agar overlay, chick embryo cell tissue cultures. Plaque-free zones are produced, thus indicating the area on paper chromatogram where the active material can be located. Using antiviral substances antibiotic W-112 and isatin-monothiosemicarbazone, W. Nile and vaccinia viruses, and 4 chromatographic solvent systems, it was possible to localize the biological activity of these antiviral agents. As little as 1 μg of antibiotic W-112 produced large plaque-free zones when paper chromatogram was applied to the overlay culture 1 hr after virus infection. When 100 μg of same material was used, large plaque-free areas resulted even when the paper was applied 46 hr after virus infection. Isatin-monothiosemicarbazone at 50 μg level not only produced a plaque-free zone but also produced a yellow spot on paper chromatogram that coincided exactly with the zone of antiviral activity.

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Reserpine and Thyroid Activity in Fowls.* (25818)

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Tranquilizers have been suggested for use with poultry because of their stress ameliorating properties with possible beneficial effect on egg production, growth and feed efficiency(1, 2). Data are available, however, indicating that reserpine has an anti-thyroid effect(3,4). If this were true of poultry, administration of reserpine would then be contraindicated for purposes of sustained tranquilization where normal thyroid function is desired. Since data are extremely limited, it was of interest to study effects of reserpine in fowls using direct measurements of thyroid function *viz.*, thyroidal-I^{131} release rate and thyroxine secretion rate.

Materials and Methods. Adult New Hamp-

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TABLE I. Effect of Varying Levels of Reserpine on Net Release of I^{131} (k' '4) from Thyroid Gland and Body Weight in Adult New Hampshire Chickens. 10 chickens/series.

Reserpine dose, μg/100 g/day	I^{131} release rate with Tapazole (k' '4)	I^{131} release rate with Tapazole and reserpine	% change in body wt during reserpine administration
10	.0079 ± .0001	.0081 ± .0002*	+ 1.1 ± .423
20	.0092 ± .0009	.0090 ± .0004	− 1.0 ± .321
30	.0101 ± .0018	.0072 ± .0009†	− 8.2 ± .689†
50	.0089 ± .0004	.0044 ± .0008‡	−15.4 ±1.826‡
100	.0099 ± .0010	.0029 ± .0001‡	−18.0 ±2.101‡

* Stand. error. † Significant at $p < .05$. ‡ Significant at $p < .01$.

shire chickens were used, subjected to diurnal temperature variations. Pigeons were housed in chamber at 78 ± 1°F. 50-75 μc I^{131} were injected subcutaneously into chickens and 24 hours thereafter external thyroid counts were made by placing birds in weighing funnel(5). In pigeons, 20 μc I^{131} were injected subcutaneously and thyroidal-I^{131} measurements were made at 24-hour intervals by placing pigeon in aluminum funnel with thyroid region approximately 15 cm over scintillation probe (6). *Determination of thyroidal-I^{131} release rate.* Thyroidal-I^{131} release rate was determined in 5 groups of 10 adult New Hampshire chickens. Daily thyroid counts were made for 11 days commencing 24 hours after I^{131} injection. Tapazole was administered as 0.1% of ration to prevent recycling of I^{131} from metabolized hormone-I^{131} which would otherwise mask true release of thyroidal-I^{131}. Beginning 6 days after I^{131} injection, birds in each group received 5 daily subcutaneous injections of varying amounts of reserpine, 10, 20, 30, 50, or 100 μg/100 g body weight/day. Thyroidal-I^{131} release curves were constructed by plotting per cent dose against time in days. Hourly rate of release of thyroidal-I^{131} (k' '4) was calculated from slope of I^{131} release curve (7). *Determination of feed intake and body weight in pigeons.* Pigeons were housed in individual cages wrapped with paper, except at top, to prevent wastage of feed. Feces was separated from feed every day before computing amount of feed consumed by individual pigeon. Pigeons were weighed daily and at least 12 days were allowed to elapse before being included in investigation. *Determination of thyroxine secretion rate (TSR).* 15-18 adult common pigeons comprised a group and were injected with citric acid (1 μg/day) as a placebo, or reserpine in varying amounts (5, 10, 15, or 30 μg/100 g body weight/day) 48 hours after I^{131} injection until TSR was estimated. Seven days after I^{131} injection, pigeons were injected with 0.25 μg 1-thyroxine/100 g body weight for 2 consecutive days and dosage was increased at 0.25 μg increments, each dose being administered for 2 days with thyroid count made on day of each increase. TSR was determined by plotting per cent previous count at each dosage, and dose which prevented further thyroidal-I^{131} release in each pigeon (95-100% of previous count) was estimated as its TSR.

Results. Rate of release of thyroidal-I^{131} (k' '4) was reduced in chickens with reserpine levels of 30 μg or above/100 g body weight (Table I). Body weight losses followed a similar pattern beginning at this level. All birds were quite inactive and decreased food consumption was evident.

Average TSR of pigeons began to decline at levels of 15 μg and above/100 g body weight/day (Table II), but body weight depression also was more pronounced at these levels. At 5 and 10 μg level, TSR was similar to citric acid-injected controls and, correspondingly, body weight loss was not marked. When feed intake was restricted to 66% of controls, TSR

TABLE II. Effect of Varying Levels of Reserpine on Thyroxine Secretion Rate (TSR) and Body Weight in Pigeons.

Reserpine, μg/100 g/day	No. of pigeons	TSR, μg/100 g/day	% change in body wt during reserpine admin.
(Controls)	15	1.26 ± .13*	− 1.1 ± .41
5	16	1.29 ± .12	− 2.0 ± .62
10	18	1.20 ± .16	− 4.2 ± .79
15	15	1.04 ± .09	− 9.3 ± .92†
30	16	.82 ± .02†	−18.3 ±1.02†

* Stand. error. † Significant at $p < .01$.

TABLE III. TSR of Pigeons at Various Levels of Feed Intake.

Treatment	No. of pigeons	TSR, μg/100 g/day	Body wt loss, %
Control (15.5 g/day)	15	1.15 ± .43	5.5 ± 1.0
Group I (66% of control)	18	.85 ± .19	12.0 ± 1.7
Group II (33% of control)	16	.72 ± .12*	15.3 ± 1.9*
Group IV (20% of control)	15	.51 ± .11*	23.0 ± 2.2*

* Significant at $p < .01$.

was depressed 27%, and at 67% and 80% less feed, TSR depression was 37% and 54%, respectively (Table III). At levels of reserpine above 10 μg/day feed consumption and body weight were severely depressed (Table IV). While 60% depression in feed intake and 13.1% body weight loss was observed at 15 μg reserpine level, there was a doubling of body weight loss at 30 μg level (Table IV).

Discussion. Administration of reserpine up to 20 μg/100 g in chickens did not alter release rate of organically bound I^{131}, whereas higher levels produced an inhibitory effect on I^{131} release both in individual and in group comparisons. Coincidentally, loss in body weight followed a similar pattern, *i.e.*, loss occurring only in groups where I^{131} release was also reduced. It was suggestive, therefore, that inanition played more than a passive role in slowing down rate of release of I^{131}. If lower levels of reserpine did reduce thyroid activity, then a certain degree of hypothyroidism would tend to favor fat deposition and cause increase in body weight rather than decrease. Such a situation was never observed. Nevertheless, TSR determination is believed to be most satisfactory index of thyroid function(8). However, observations in pigeons were similar to that in chickens, thyroid inhibition being observed where loss in body weight was observed (Table II) or where feed was restricted (Table III). TSR was reduced only when body weight loss exceeded 9% (Tables II and III). TSR of restricted fed pigeons (Table III) paralleled decreased TSR found at 15 μg/100 g (and above) reserpine level (Table II). Body weight loss and depression in feed intake in pigeons administered graded levels of reserpine (Table IV) paralleled those in whom decreased TSR was noted with increasing levels of reserpine (Table II). When body weight loss was less than 9% and depression in feed intake did not exceed 5%, TSR was not affected, (*i.e.*, when 5 and 10 μg reserpine was administered). No level of reserpine produced thyroid inhibition without depressing feed intake and body weight.

Combined observations in pigeons and chickens indicate that physiological levels of reserpine have no effect on thyroid function in fowls. Thyroid inhibition occurring at relatively high levels of reserpine is very likely due to decreased feed intake. Inanition has been shown to depress thyroid activity(9). Observations of decreased thyroid size and increased cholesterol levels (a measure of inhibition of thyroid function) in serpasil-fed birds with concomitant depression in body weight(10) lends further support to inanition postulation. Observation that reserpine inhibits thyroid activity in some species only indicates species difference. In fowls, physiological levels of reserpine do not have any influence on thyro-pituitary axis.

Summary. Reserpine at levels of 30 μg/100 g/day and above depressed thyroidal-I^{131} release rate in chickens and caused body weight losses. At 15 and 30 μg/100 g/day, thyroxine secretion rate (TSR) was reduced

TABLE IV. Effect of Varying Levels of Reserpine on Body Weight and Feed Intake in Pigeons.

Treatment	No. of pigeons	Depression in feed intake (%)	Body wt loss (%)
Control (citric acid inj.)	18	5.1 ± .98	3.2 ± .82
Group I, 5 μg reserpine/100 g/day	15	6.2 ± 1.0	6.1 ± 1.0
" II, 10 μg "	16	10.0 ± 1.3	5.5 ± .79
" III, 15 μg "	15	60.0 ± 5.5*	13.1 ± 1.8 *
" IV, 30 μg "	16	80.0 ± 6.8*	26.2 ± 3.6 *

* Significant at $p < .01$.

in pigeons with depression in body weight and feed intake. No level of reserpine was found which would inhibit TSR without depressing feed intake. It is suggested that depression of thyroid activity at higher levels of reserpine is secondary to depression in food intake.

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Factor VIII (Antihemophilic Factor) and Factor V (Proaccelerin) Activity of Platelets.* (25819)

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Roskam postulated that normal platelets contain on their surface plasma coagulation factors. He used the expression "plasmatic atmosphere of platelets" to describe this phenomenon(1). Mann *et al.*(2) and later Ware *et al.*(3) showed that normal platelets have factor V activity. Experiments of Hjort *et al.* (4) supported the theory that factor V was adsorbed on the platelet surface. Bounameaux(5) demonstrated the multiplicity of plasma factors adsorbed on platelet surface. The present paper is concerned with 1) demonstrating that normal platelets contain material with factor VIII or factor V activity in quantities sufficient to correct to normal certain diagnostic tests for study of blood from patients with defects of these factors; 2) demonstrating that platelets separated from blood of patients with plasma deficiency of factor VIII or factor V present a defective activity of the corresponding factor; 3) describing some properties of these active factors of platelets.

Materials and methods. Materials and methods used for diagnostic purposes have been described(6,7). All plasma samples used were obtained by mixing blood with 3.8% sodium citrate in proportion of 1:9. Platelets were separated by differential centrifugation and washed 10 times with normal saline. Concentration of normal platelet suspension was then made equal to that of patient's platelets, following a semivolumetric method previously described(6). A 1% suspension v/v was used unless otherwise indicated. When lyophilized platelets were used, weight of dry material was equivalent to that of fresh elements contained in 1% concentration. Platelet suspensions were used in various coagulation mixtures according to technic of "thromboplastin generation test" (TGT), described by Biggs and Douglas(8). Our results are, however, reported in 1 figure (called "prothrombin activation index" or PAI)(6) rather than with a curve. Normal PAI is 9.8 $\pm$ 2.9 (2 standard deviations). The systems used are described with specific experiments. Other coagulation mixtures were tested following the basic technic of "prothrombin time" (one-stage method). Systems used are described with specific experiments. Tissue thromboplastin was a commercial preparation obtained from rabbit brain.†

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