compounds, more closely related derivatives of DDT would have to be used as a hapten, i.e., Kelthane [1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol].

The interpretation of immunological results with DDA are made difficult by its low solubility in water. Proper choice of solvents enabled us to get what appear to be meaningful results.

The lack of stability of the hapten specific antibodies cannot be explained at this time. As the antibodies formed against both types of insecticide derivative showed this lability, the problem deserves closer study, so that reasons for the lack of stability and the optimal conditions for the preservation of the sera can be determined.

The transient immune response against DDA-FBN and MLT-FBN is similar to that reported against insulin (5). We cannot explain at this time the reason for the transitory nature of the antibodies.

The immunological approach to the assay of pesticides could be extended to other pesticides and herbicides, with particular emphasis on those which lend themselves to covalent bonding with proteins, i.e., 2,-4-D[(2,4-dichlorophenoxy)acetic acid].

Summary. Antibodies against conjugates of DDA and Malathion half ester with bovine fibrinogen were produced by sensitizing rabbits with these conjugates. No direct reaction was found against DDA or Malathion, but these compounds inhibited the hemagglutination of their respective conjugate. The antihapten antibodies were transitory, and their activity was lost during storage, unless frozen and kept at very low temperatures. Due to the hydrophobic nature of DDA, organic solvents were used in the precipitin and hemagglutination tests involving this compound.

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Antithyroid Effects of 3-Amino-1, 2, 4-triazole* (33367)

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Astwood and associates (1) tested a large number of compounds for their antithyroid activity in 1945. Among these were 3-mercapto-1,2,4-triazole and related compounds. The triazole compounds exhibited some antithyroid activity but were less active than thiouracil, the standard for comparison at that time. In recent years, another triazole compound, 3-amino-1,2,4-triazole (ATZ), has become available and has gained importance as a herbicide, plant growth inhibitor, and defoliant. These effects are probably the result of reduction in chlorophyll (2) and riboflavin (3) content and in catalase activity in the plant (2). In rats, ATZ reduces hepatic and renal catalase and, to a lesser extent, hepatic peroxidase but not erythrocytic peroxidase (2,4).

Alexander (5) called attention to the selective action of ATZ toward tissue hydroperoxidase and noted that the compound inhibits ^{131}I uptake and the organic binding of ^{131}I without affecting the iodide "trap" in rats. Pitt-Rivers (6) confirmed these effects.

The present study extends the observations

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on the antithyroid activity of ATZ in rats and compares its effects with those of propylthiouracil (PTU) and potassium perchlorate ($KClO_4$).

Methods. One hundred forty weanling, female, Sprague-Dawley rats were randomly divided into four groups. These groups received either a control diet (Ken-L-Ration) (about 0.25 μg of iodine/g at the time used) or the control diet plus 1.0% (w/w) $KClO_4$, 0.1% ATZ, or 0.1% PTU. The body weights were determined at 3–7-day intervals for 83 days and then all animals were placed on the control diet.

At various intervals, animals from each group were killed. The thyroids from these animals were removed and weighed. Total iodine determinations (7, 8) were made on each gland. On days 11 and 83 one gland from one rat from each group was prepared for histologic examination.

To study intrathyroid metabolism of iodide, a group of 44 rats weighing 100–150 g was given a diet relatively high in iodide (Rockland mouse/rat diet). Rats fed this ration had 24-hr thyroid uptakes of ^{131}I of 3–5% of the dose. Therefore for 6 days before the animals were used, a diet relatively low in iodide (Remington, General Biochemicals) was fed (after this regimen, 24-hr thyroid uptake of ^{131}I ranged from 8 to 12% of an administered dose of 25 μCi). At the time of the determination of the 24-hr uptake of ^{131}I, the animals were divided into control and treated groups. In the control group, four rats were anesthetized with ether and the thyroid glands were removed for hydrolysis and ^{131}I counting. At 24-hr intervals thereafter for 5 days, four rats from the control group were thyroidectomized and the glands were analyzed. The remaining 20 rats served as a treated group and were given 10 mg of ATZ in 20% gelatin by subcutaneous injection at the time of the determination of the 24-hr thyroid uptake of ^{131}I. The ATZ (0.1% w/w) was added to the powdered Remington diet. At 24-hr intervals after institution of treatment with ATZ, four animals were killed with ether and the thyroid glands were removed and analyzed.

Each thyroid gland was ground in a glass homogenizer with 1.0 ml of cold 0.11 *N* sodium chloride–0.04 *N* sodium bicarbonate solution. The suspension was centrifuged, and 0.3 ml of the supernate was added to 0.2 ml of a 2.0% suspension of *Streptomyces griseus* protease (pronase, Calbiochem) in the $NaCl–NaHCO_3$ solution. To this mixture was added 20 μl of a 1.0% suspension of PTU, 10 μl of 0.1 *M* manganese sulfate, and 2 drops of toluene. This preparation was incubated for 2 hr at 38°. A 25 to 50-μl aliquot of each hydrolyzate was applied to 1.5-in. strips of Whatman no. 3 MM paper and developed by ascending chromatography in *n*-butanol–absolute ethanol–0.5 *N* ammonium hydroxide (BEA, 5:1:2) for about 10 hr. The chromatographic strips were analyzed for radioactivity by an automatic strip scanner using a beta detector with 4-π geometry and an integrator. Authentic standards were visualized by the use of palladium chloride stain for iodide and ferric chloride–potassium ferricyanide for the tyrosines and thyronines. Resolution of the compounds in the BEA solvent system was sufficiently good that only the one system was required for identification.

Results. The growth rate for each of the goitrogen-fed groups was less than that of the control group. The retardation of growth was greatest in the PTU group followed, in order, by the groups given ATZ and $KClO_4$. Whereas each group had an average weight of 50 g at the beginning of the experiment, the control, $KClO_4$, ATZ, and PTU groups weighed 252, 201, 159, and 144 g, respectively, at 83 days of treatment.

The effects of the various dietary regimens on thyroid weight and content of iodine are shown in Table I. The thyroid weight in the control group remained constant at 5–8 mg/100 g of body weight throughout the 106 days of observation. Significant increases in weight of the thyroid were noted by the third day of treatment in all the goitrogen-fed groups. The goiters in the $KClO_4$ group were smaller throughout the 83 days of treatment than the goiters in the ATZ and PTU groups. The goiters in the treated groups did not regress during the 23 days following cessation of treatment, probably because of the low

TABLE I. Effect of Goitrogens on Thyroid Weight (TW)[a] and Total Iodine Content (TTI).[b]

Days on diet[c]	Control		Aminotriazole		Propylthiouracil		$KClO_4$	
	TW	TTI	TW	TTI	TW	TTI	TW	TTI
3 (4)	8 (±1)	49 (±1)	16 (±6)	2 (±2)	11 (±2)	11 (±7)	12 (±3)	17 (±12)
6 (4)	8 (±1)	56 (±25)	18 (±7)	1 (±1)	27 (±5)	0.5 (±0.1)	16 (±3)	4 (±5)
11 (3)	6.8 (5.6–8.6)	53 (45–64)	37 (31–43)	0.5 (0.4–0.7)	49 (37–64)	0.4 (0.2–0.6)	27 (23–32)	0.1 (0.1–0.1)
17 (4)	8 (±1)	36 (±11)	51 (±16)	0.5 (±0.1)	38 (±3)	0.5 (±0.2)	26 (±4)	0.1 (±.04)
24 (4)	8 (±1)	29 (±12)	51 (±19)	0.5 (±0.1)	84 (±34)	0.5 (±0.2)	36 (±12)	.03 (±.01)
31 (4)	8 (±2)	55 (±22)	56 (±3)	0.6 (±0.1)	57 (±3)	2 (±3)	27 (±3)	0.2 (±0.1)
83 (3)	6 (5–8)	58 (33–69)	77 (39–120)	0.3 (0.1–0.7)	66 (53–81)	0.3 (0.2–0.4)	24 (22–31)	0.1 (0.1–0.2)
91 (2)	8 (8–8)	68 (55–80)	33 (30–66)	11 (10–12)	65 (57–72)	0.2 (0.1–0.2)	19 (12–26)	4 (2–6)
99 (2)	6 (6–6)	54 (50–57)	35 (35–35)	1 (1–2)	27 (17–38)	3 (3–3)	10 (7–13)	4 (2–6)
106 (3)	5 (5–6)	35 (24–46)	28 (20–37)	14 (10–17)	42 (40–43)	4 (3–6)	13 (11–14)	2 (2–3)

[a] Mean thyroid weight (mg/100 g of body wt.) ± SD or range.
[b] Mean thyroid total iodine ($\mu g\ I^-$/100 mg of thyroid wt.) ± SD or range.
[c] All animals were returned to the control diet on day 83. Numbers of animals in group are given in parentheses.

iodide content of the diet. In the control group, the thyroid total iodine (Table I) remained relatively constant at 29–68 μg/100 ml of thyroid weight. The total iodine in the thyroid of the $KClO_4$ group was depleted somewhat less rapidly than that of the PTU and ATZ groups but ultimately reached lower levels. As shown in Fig. 1 and expected (9), the hyperplasia in the ATZ group was not qualitatively different from that in the two other treated groups.

The components of hydrolyzates of thyroids from animals used as controls are shown in Table II. Fifty percent of the 24-hr ^{131}I level remained after 5 days. When a semilog plot was made of the remaining thyroid ^{131}I against time in days, the points fit a reasonably straight line. The biologic half-time of the ^{131}I in the glands of these control rats was 4.9 days as determined by the method of least squares. Over the 5-day period, the proportions of monoiodotyrosine (MIT), diiodotyrosine (DIT), and thyroxine (T4) in the thyroid hydrolyzates remained relatively constant. The proportion of triiodothyronine (T3) was small and variable.

The results of the same type of experiment performed on ATZ-treated rats are shown in Table III. The biologic half-life of ^{131}I was reduced to 1.3 days. The ^{131}I activity in the DIT component remained fairly constant over the 5-day period whereas the MIT component increased somewhat. The most striking change, however, was the 67% reduction in T4. This finding is similar to that previously found for PTU-fed rats (10). In the present experiments there appeared to be a gradual decrease in the T3 components, simi-

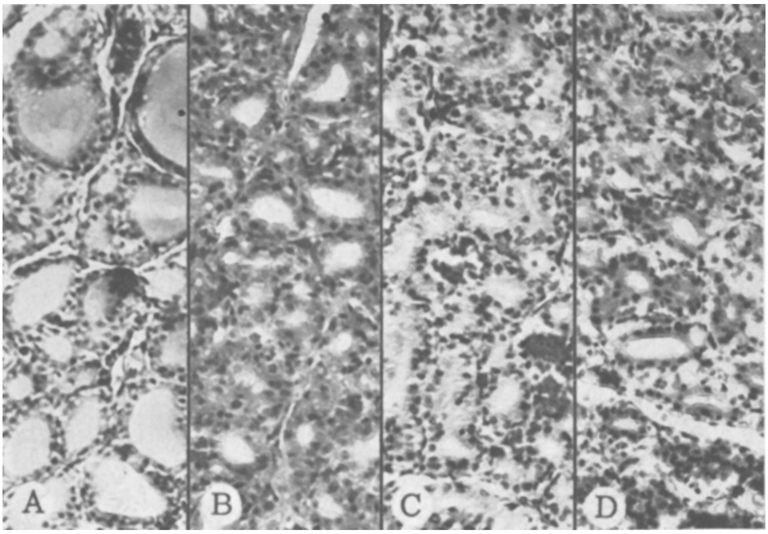

FIG. 1. Histologic sections of thyroid glands of rats fed control diet (A), or control diet plus aminotriazole (B), propylthiouracil (C), or $KClO_4$ (D). Hematoxylin and eosin; ×170.

lar to the findings for T4, but, because of the low initial level and the variability of this component in the control animals, interpretation of the finding in the ATZ-treated animals is uncertain.

Discussion. Since the molecular weight of PTU is approximately twice that of ATZ, the ATZ-treated rats received twice the moles of goitrogen in an equivalent weight of diet compared to the PTU-treated rats. Nevertheless, on the basis of weight, ATZ was as effective as PTU. Furthermore, the two compounds caused similar depletion of thyroid iodine and goiters of comparable size. However, the ATZ-treated rats gained weight more normally than did the PTU-fed group, perhaps because of the greater toxicity of PTU.

The effect of ATZ on intrathyroid metabolism of iodide in the rat was similar to that of PTU in all aspects studied. The biologic half-life of ^{131}I in the thyroids of animals treated with the former was shorter than that in animals treated with $KClO_4$ (10). Whereas $KClO_4$ depleted thyroid iodine more than did ATZ or PTU, it produced smaller goiters. Astwood and Bissell (11) have shown that, after treatment with thiouracil, most of the iodine in the thyroid is inorganic iodide. The same probably is true for the glands of rats treated with PTU or ATZ but not for those treated with $KClO_4$ which primarily affects

TABLE II. Components of Hydrolyzates[a] of Thyroid Glands from Control Animals.[b]

Group	Time after ^{131}I (hr)	Thyroid ^{131}I remaining (% of initial)	^{131}I activity in components of thyroid hydrolyzates (%)					
			MIT	DIT	T3	T4	I^-	Origin
0	24	100	30 ± 2	41 ± 3	2.3 ± 1.5	18 ± 1	2 ± 2	8 ± 1
1	48	74	34 ± 2	41 ± 4	0.7 ± 0.5	16 ± 3	2 ± 2	7 ± 1
2	72	66	29 ± 3	49 ± 3	0.1 ± 0.2	15 ± 1	1 ± 1	8 ± 1
3	96	57	35 ± 6	42 ± 4	0.6 ± 0.5	16 ± 3	1 ± 1	8 ± 2
4	120	49	34 ± 4	40 ± 5	1.3 ± 0.5	17 ± 2	1 ± 1	7 ± 2
5	144	49	31 ± 5	46 ± 3	0.4 ± 0.4	16 ± 3	1 ± 1	6 ± 2

[a] *S. griseus* protease digestion.
[b] Means ± SD; 4 animals/group.

TABLE III. Components of Hydrolyzates of Thyroid Glands from Aminotriazole-Treated Animals.[a]

Group	Treatment time	Thyroid ^{131}I remaining (% of initial)	^{131}I activity in components of thyroid hydrolyzates (%) MIT	DIT	T3	T4	I⁻	Origin
0	0[b]	100	30 ± 2	41 ± 3	2.3 ± 1.5	18 ± 1	2 ± 2	8 ± 1
1	24	52	35 ± 4	40 ± 5	2.0 ± 2.0	15 ± 5	2 ± 3	7 ± 2
2	48	34	34 ± 2	47 ± 1	0.8 ± 0.3	10 ± 2	2 ± 1	7 ± 1
3	72	19	38 ± 2	44 ± 3	0.2 ± 0.4	9 ± 3	2 ± 1	8 ± 1
4	96	9	39 ± 3	41 ± 2	0.2 ± 0.4	8 ± 1	3 ± 2	8 ± 2
5	120	9	43 ± 5	44 ± 3	0	6 ± 3	2 ± 2	6 ± 1

[a] Means ± SD; 4 animals/group.
[b] Equivalent to 24 hr after ^{131}I injection.

iodide concentration. Alexander and Wolff (12) have called attention to the disparity in size of goiters produced by PTU and $KClO_4$. The present study confirms this and shows that the smaller goiters of $KClO_4$ are not due to lack of iodine depletion in the gland.

ATZ, like PTU (10), causes depletion of T4 relative to the iodotyrosines in thyroid hydrolyzates, whereas $KClO_4$ does not (10). The latter finding may be interpreted to mean that ATZ not only interferes with the initial iodination of tyrosine within thyroglobulin but also inhibits the coupling of iodotyrosines to form iodothyronines, as originally suggested by Pitt-Rivers (13) in the case of thiouracil. However, these effects of PTU and ATZ do not explain their enhancement of the rate of release of ^{131}I from the thyroid as compared to $KClO_4$ (10). A likely explanation for this difference is that ATZ and PTU augment the effect of thyrotropin on the thyroid. Albert and associates (14) and Halmi and Spirtos (15) have shown that PTU does augment the effect of thyrotropin on the thyroid. Since ATZ has been shown to be similar in its action on thyroid function in all other respects, it is likely that it also would have a thyrotropin-augmentative effect on the thyroid.

Summary. The effects of 3-amino-1,2,-4-triazole (ATZ) on weight, content of iodine, and histology of the thyroid in rats were determined and compared to the effects of propylthiouracil (PTU), and $KClO_4$. In an 83-day period of treatment, ATZ and PTU produced goiters of comparable size. The $KClO_4$ treatment resulted in slightly greater depletion of iodine in the thyroid, smaller goiters, and less thyroxine depletion of the thyroid. The effect of ATZ treatment on intrathyroid metabolism of iodine in rats was studied and compared to similar studies in rats without treatment. In the ATZ-treated group, there was a progressive decrease in T3 and T4 and a slight increase in the proportion of MIT. The biologic half-life of ^{131}I in the thyroid of the control and treated groups was 4.9 and 1.3 days, respectively. These findings are similar to those found previously for PTU and show that ATZ, like PTU, not only interferes with organification of iodine but also inhibits the coupling of iodotyrosines to form iodothyronines.

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Yaba Tumor Virus. I. Studies on Pathogenesis and Immunity* (33368)

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Subcutaneous noncapsulated tumors of histiocytic origin (histiocytomas) were first observed in 1957 among rhesus monkeys kept in open air cages at Yaba, Nigeria (1). In subsequent studies, the etiological agent was found to be a member of the poxvirus group (2) and inoculation of human volunteers with the virus produced similar tumors (3).

Yaba tumors, whether occurring naturally or induced experimentally in monkeys regress spontaneously within 1–2 months; regression is apparently due to an *in vivo* cytopathic effect of the causative agent (2, 4). While the tumors are progressing, specific antibodies develop and appear to have little if any effect on established tumors (5). Cross resistance experiments indicated no immunogenic relationship between Yaba tumor virus on one hand, and vaccinia, monkey pox, and orf viruses on the other (2, 6).

In the present experiments we studied pathogenic and immunologic responses of rhesus monkeys to Yaba tumor virus; the antigenic relationship between Yaba, vaccinia, and monkey pox viruses; and the development of viral antigen in tumor cells as monitored by the complement fixation and fluorescent antibody techniques.

Materials and Methods. Viruses. Yaba tumor virus[2] was obtained from Dr. David S. Yohn, Roswell Park Memorial Institute, Buffalo, New York. The virus was passed twice in rhesus monkeys by the subcutaneous route of inoculation and a large stock of tumors in 5- to 10-gram quantities were stored frozen at —70°. Monkey pox virus[2], as infected cell culture fluid, was obtained from Dr. Preben von Magnus, Staten Seruminstitut, Copenhagen. The vaccinia virus was derived from a commercial (Wyeth) smallpox vaccine preparation.

Monkeys. Young adult rhesus or cynomolgus monkeys lacking preexisting Yaba virus antibodies, as determined by the CF test, were used for all Yaba virus infections. Monkeys were inoculated with Yaba virus either by subcutaneous (s.c.) or intravenous (i.v.) routes. Inoculated monkeys were kept

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