THE EFFECT OF 6-MERCAPTOPURINE AND AMINOPTERIN ON EXPERIMENTAL IMMUNE THYROIDITIS IN GUINEA PIGS*

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Plates 81 to 84

(Received for publication, June 25, 1963)

In 1958 Schwartz *et al.* first described the suppressive effect on antibody formation in rabbits of the purine antagonist 6-mercaptopurine (1). Stimulated by this finding a number of investigators have studied the effect of different antimetabolites on antibody formation (2–4) and on delayed hypersensitivity (5, 6) in various animal species. These findings are not only of theoretical interest, but have become of practical importance in homotransplantation (7–9), in the treatment of autoimmune disorders in man (10, 11), and in experimental animals (12).

The action of antimetabolites on the immune response differs from species to species. 6-mercaptopurine (6-MP) and aminopterin have both been reported to inhibit delayed hypersensitivity in the guinea pig (5, 13). In contrast, antibody formation in this animal was only suppressed by aminopterin (13, 14). Thus the mechanism of action of these two compounds on the immune response in guinea pigs appears to be different. For this reason it seemed of interest to study and compare the effect of these two antimetabolites on an experimental immune disease in this species.

In the present study immune thyroiditis was selected as a suitable experimental immune disease in the guinea pig, because the responsible antigen is well known and antibody formation as well as delayed hypersensitivity against thyroglobulin can be easily determined.

The antimetabolites were administered during the immunization phase in order to study their effect on the immune response as well as on the changes in the thyroid gland. In addition, the antimetabolites were given to animals which

*This work was supported by grants No. AM-3777 and AM-4819, United States Public Health Service.

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had been immunized and which had already developed thyroiditis, since it seemed important to determine whether the course of an immune disease could be altered by these agents.

Materials and Methods

Animals.—Female Hartley strain albino guinea pigs weighing 230 to 270 gm were used throughout all experiments. They were maintained on laboratory chow and water *ad libidum*. The control animals gained weight, weighing 300 to 350 gm at the end of the experiments.

Immunization.—Thyroid glands of normal guinea pigs were collected and frozen at −20°C to a block of thyroid tissue. This block in the frozen state was cut with a razor blade into fine sections. The sections were extracted overnight with 3 times their weight of 0.15 M NaCl. The extract was centrifuged at 4°C at 3000 RPM for 15 minutes. The clear supernatant was used as thyroid extract and stored at −20°C until used. The protein content of the extract was determined by the method of Folin-Ciocalteu. The preparations usually contained 20 to 30 mg protein per ml. Purified thyroglobulin was prepared according to the method of Derrien *et al.* (15), modified by Roitt and Doniach (16).

In the first series of experiments 500 μg of purified thyroglobulin was used for the immunization, while in all the following experiments 750 μg of thyroid extract protein served as antigen. The latter was as effective as purified thyroglobulin, but fewer thyroid glands were necessary to prepare the antigenic substrate.

The thyroid extract was diluted with 0.15 M NaCl and emulsified in an equal volume of complete Freund adjuvant (Difco Laboratories, Inc., Detroit) to a final concentration of 750 μg protein thyroid extract per 0.2 ml. 0.1 ml of this mixture was injected in each front foot-pad on day 0 and the same amount was injected on day 4 in the hind foot-pads.

Skin Testing.—The animals were skin-tested with 50 μg guinea pig thyroglobulin in 0.1 ml 0.15 M NaCl. The reaction was read 6 and 24 hours later. Infiltration of the skin 24 hours after injection of the thyroglobulin measuring 2 × 2 mm or more was classified as a positive delayed reaction. Induration of the skin was taken as indication of positive delayed hypersensitivity since 3 of 20 non-immunized animals showed small erythematous areas 24 hours after skin testing with thyroglobulin. The diameter of erythema in the immunized animals was not much larger than that of non-immunized animals.

Serum Antibodies.—At the end of the experiments, the animals were exsanguinated and the blood allowed to clot at room temperature for 2 hours before separation of the serum. The sera were heat-inactivated at 56°C for 30 minutes and absorbed with ⅛ volume of packed washed sheep erythrocytes at 4°C overnight.

A tanned cell hemagglutination test was performed according to a modified method of Heller *et al.* (17). Sheep red cells (Probio Inc., New York) in Alsever's solution were washed 3 times with phosphate buffer pH 8 described in reference 17. A 50 per cent concentration of cells was treated with an equal volume of tannic acid diluted 1:20,000 for 10 minutes at 37°C. The tannic acid (Mallinckrodt, analytical reagent) was prepared daily in buffer. The tanned cells were centrifuged, washed once with buffer, and reconstituted to a 20 per cent solution for coating with the antigen. Equal parts of 20 per cent tanned sheep erythrocytes and a solution containing 2 mg thyroglobulin per ml buffer were incubated for 30 minutes at 37°C in a water bath. After coating with the antigen the sheep cells were washed 3 times with buffer and reconstituted in buffer containing 0.5 per cent normal guinea pig serum to a final concentration of 0.5 per cent erythrocytes. 2-fold dilutions were made in the micro titrator of Takatsy (Danube International Trade Corp., New York) starting with a first dilution of 1:16, and using 0.05 ml antiserum and 0.05 ml thyroglobulin-coated sheep erythrocytes. The plates were allowed to stand overnight at room temperature before reading the

agglutination titer. Positive and negative control sera were included in each test as well as controls with uncoated tanned erythrocytes to exclude a non-specific or spontaneous agglutination.

The sera were further analyzed for precipitating antibodies in the agar double diffusion technique in petri dishes using 0.5 per cent agar in 0.15 M NaCl.

Histological Examination.—Immediately after sacrificing the animals both lobes of the thyroid were removed and fixed in 10 per cent formalin. 3 to 4 sections through the largest part of the thyroid were stained with hematoxylin and eosin. In almost all cases both lobes showed a similar degree of thyroiditis; when different degrees of thyroiditis were observed the stronger reaction was taken as the result. The degree of thyroiditis was graded arbitrarily from 1+ to 4+ according to the extent of infiltration of the cross-section of the thyroid gland: Negative, normal thyroid tissue (without or only one small perivascular infitration); 1+, 2 to 4 small infiltration areas; 2+, infiltration of 25 to 50 per cent; 3+, infiltration of 50 to 75 per cent and 4+, complete infiltration of the thyroid tissue.

White Blood Counts.—0.025 ml blood was taken from the retroorbital plexus at regular intervals with a calibrated heparinized glass pipette and the blood was transferred into 0.475 ml Turck solution and immediately mixed to obtain a homogeneous suspension. The leucocytes were counted and differenciated into mononuclear and polymorphonuclear cells in the Thoma WBC chamber.

6-Mercaptopurine.—6-MP was kindly supplied by Dr. G. H. Hitchings, Burroughs, Wellcome, and Company, Inc. Tuckahoe, New York. 50 mg of 6-MP per ml of physiological saline was dissolved with the minimal amount of 10 per cent NaOH necessary to completely dissolve the drug. All preparations were made up daily. 6-MP was administered intraperitoneally or intramuscularly in the muscle of the hind legs. The most effective dosage of 150 mg/kg intraperitoneally caused a mortality of 75 per cent of the animals if given daily, however 5 injections weekly reduced the toxic effect to about 25 per cent mortality. Since preliminary experiments had shown that daily injections did not result in a better effect than 5 injections weekly, the latter treatment schedule was used throughout all experiments. Treatment with 150 mg/kg intraperitoneally resulted in an average weight loss of 20 per cent.

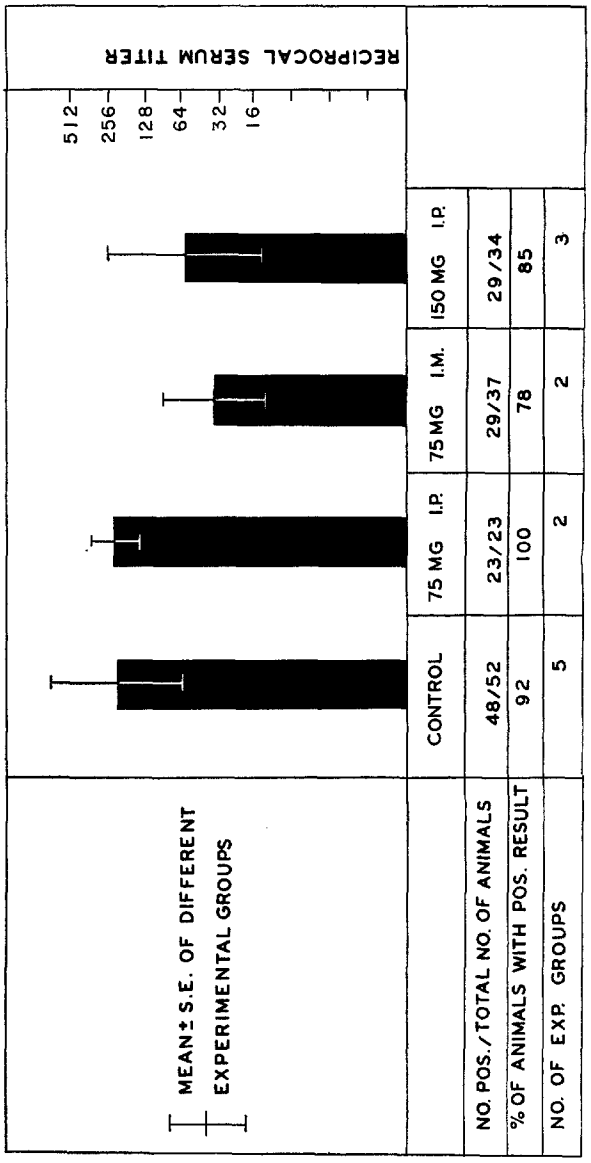
Aminopterin.—Aminopterin was kindly supplied by Dr. Ruegsegger, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, as methotrexate vials. Aminopterin was given daily intraperitoneally in dosages of 5 mg and 10 mg/kg which were well tolerated by all animals; they gained weight almost at the same rate as the control animals.

Treatment Schedule.—The time of administration of 6-MP or aminopterin was performed according to seven different schedules shown in Table I.

RESULTS

Immune Response and Incidence of Thyroiditis in Control Animals.—Delayed hypersensitivity and antibodies against thyroglobulin as well as the appearance of thyroiditis were determined repeatedly in a total of 101 control animals which have been immunized with two injections of thyroid extract on days 0 and 4 as indicated under Material and Methods.

Out of 11 animals skin-tested on day 10, 5 (45 per cent) showed a positive delayed skin reaction. The 11 animals were exsanguinated on day 11 for antibody determination and histological examination of the thyroid gland. 4 of these animals exhibited serum antibodies in a titer of 1:16 and higher; 3 animals showed mild thyroiditis.



	CONTROL	75 MG I.P.	75 MG I.M.	150 MG I.P.
NO. POS./TOTAL NO. OF ANIMALS	48/52	23/23	29/37	29/34
% OF ANIMALS WITH POS. RESULT	92	100	78	85
NO. OF EXP. GROUPS	5	2	2	3

TEXT-FIG. 1. Mean values for serum antibody titer of control animals and guinea pigs treated with 6-MP in different dosages (75 mg/kg and 150 mg/kg) and route of administration (intramuscular and intraperitoneal), according to schedule I.

52 animals were skin-tested on day 20 and bled on day 21. The incidence of delayed hypersensitivity was 65.5 per cent and the incidence of antibodies 92 per cent. Thyroiditis was present in 82 per cent of the animals.

20 animals were examined 30 days after immunization. 18 (90 per cent) showed a positive delayed skin reaction; 18 animals had serum antibodies. Thyroiditis was found in 16 (80 per cent) animals.

18 animals were skin-tested at day 40 and bled on day 41. 12 animals (66.6 per cent) showed a positive delayed skin reaction and 2 animals showed an immediate reaction of the Arthus type. All 18 animals had severe thyroiditis.

The average and standard deviations of serum antibody titer, diameter of skin infiltration, and degree of thyroiditis of the different control groups are

TABLE I

Treatment Schedules

Schedule No.	Days of treatment with antimetabolites*					Day of skin test	Day of termination of experiment
	−10 to −1	0 to 9	10 to 19	20 to 29	30 to 39		
I	0	+	+			20	21
II	+	0	0			20	21
III	0	+	0			20	21
IV	0	+	0	0		30	31
V	0	0	+			20	21
VI	0	0	0	+		30	31
VII	0	0	0	+	+	40	41

+, administration of antimetabolites; 0, no administration of antimetabolites.

* Days 0 and 4, days of injection of antigen in all treatment schedules.

graphically registered, together with the data of the corresponding treated animals, in Text-figs. 1 to 10.

The development of immune thyroiditis in the control animals is characterized by a change in the thyroid epithelium cells and by a round cell infiltration The epithelium cells of the normal guinea pig thyroid appear flat in cross-section (Fig. 1). These epithelium cells become swollen in animals developing thyroiditis and appear cuboidal (Fig. 2.). Simultaneously, inflammatory mononuclear cells appear in the perivascular spaces, mainly histiocytes and lymphocytes, extending gradually until the whole thyroid gland becomes infiltrated (Fig. 2). The graduation of the severity of thyroiditis has been based on the extent of infiltration with round cells as outlined under Material and Methods.

Immune Response and Incidence of Thyroiditis in Animals Treated with 6-MP. —A total of 204 guinea pigs were treated with 6-MP. All seven schedules of drug administration (Table I) were used.

Treatment according to schedule I: The effect of 6-MP on antibody formation according to schedule I for different dosages and route of administration is sum-

marized in Text-fig. 1. No significant suppression of antibody formation to thyroglobulin was found by all dosages used. In two out of three experiments with the highest dosage of 150 mg/kg intraperitoneally the average antibody titer was found slightly higher than in the control animals immunized simultaneously. A similar result was obtained with the agar diffusion technique, where 61 per cent of the control animals showed precipitating antibodies compared with 55 per cent (75 mg/kg intraperitoneally) and 45 per cent (150 mg/kg intraperitoneally) of the treated animals.

Delayed hypersensitivity was not significantly influenced by 75 mg/kg 6-MP given intraperitoneally, but markedly depressed by the same dosage given intramuscularly. (Text-fig. 2). 150 mg/kg 6-MP given intraperitoneally was equally effective as 75 mg/kg given intramuscularly; only 33 per cent of these animals showed a positive skin test in contrast to 65 per cent in the control animals.

Thyroiditis was depressed by all dosages used; animals treated with the highest dosage (150 mg/kg) developed thyroiditis in only 29.5 per cent in contrast to 82 per cent in the control animals (Text-fig. 2). No significant difference in the effectiveness in suppression of thyroiditis was found between the intramuscular and intraperitoneal administration of 6-MP.

The histological pattern of the thyroid of the 6-MP treated animals which developed thyroiditis was qualitatively similar to that of control animals although the intensity of inflammation was usually lower.

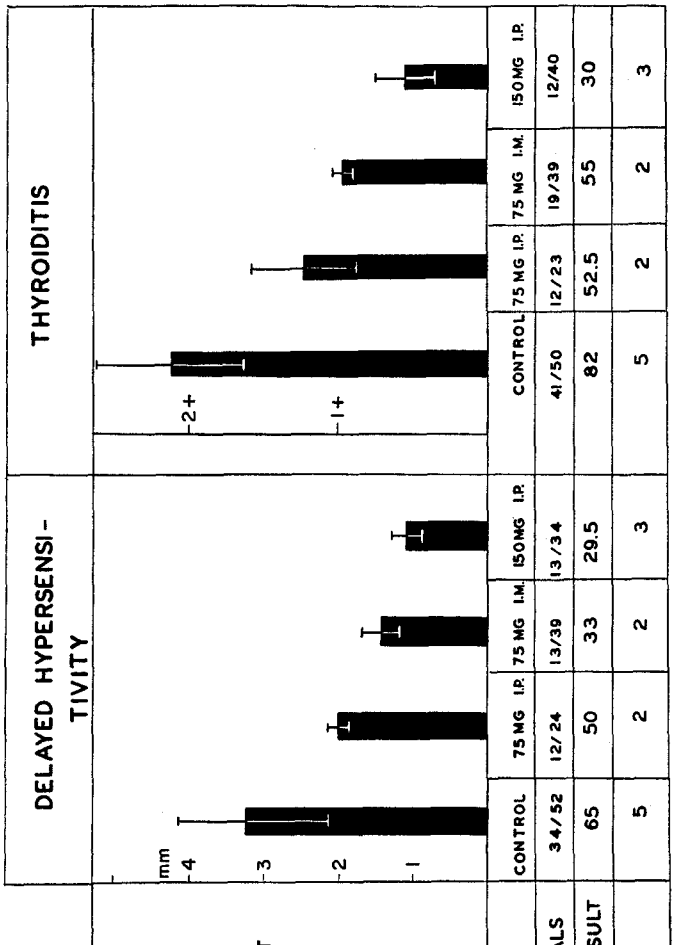
Among control animals exhibiting a positive skin test 80 per cent showed thyroiditis. In the guinea pigs treated with 150 mg/kg 6-MP this coincidence was found in 50 per cent of the animals.

One group of 11 animals was treated with only 3 weekly intraperitoneal injections of 6-MP (150 mg/kg). This treatment resulted in an equal suppression of thyroiditis (3 of 11 animals showed mild thyroiditis), however, no influence was observed on the skin test.

Among the various doses and routes of administration, 150 mg/kg given intraperitoneally was found most active. For this reason, 6-MP was given in this dose intraperitoneally to all animals in the subsequent studies.

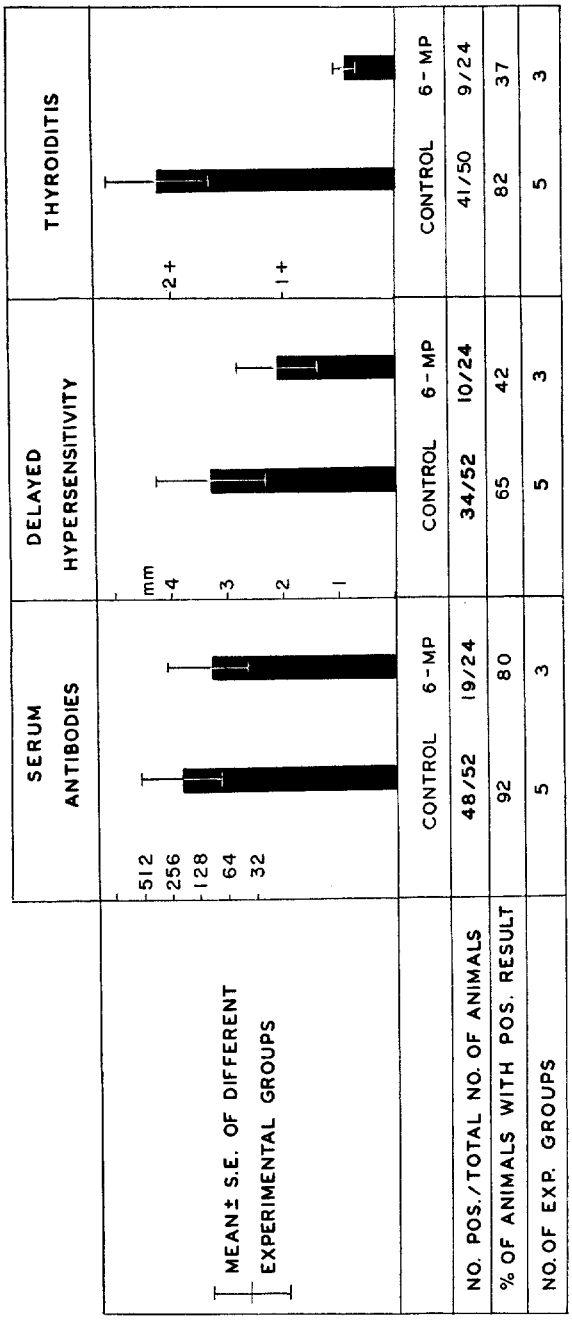
Treatment with 6-MP preceding immunization: Pretreatment with 6-MP of 11 animals preceding immunization with thyroid extract according to schedule II resulted in no significant change of the immune response and development of thryoiditis (73 per cent antibodies, 63.5 per cent positive skin test, 73 per cent thyroiditis).

Treatment in the early phase of immunization: Treatment during the first 10 days of the immunization course (schedule III) was equally effective in depressing thyroiditis as 6-MP given during the entire period of immunization, however delayed hypersensitivity was less depressed; again no influence on antibody formation was observed (Text-fig. 3). 11 such treated animals were examined 20 days after discontinuing of 6-MP treatment (schedule IV) on day 31: The incidence of thyroiditis was not different from that of control animals examined



	CONTROL	75 MG I.P.	75 MG I.M.	150MG I.P.	CONTROL	75 MG I.P.	75 MG I.M.	150MG I.P.
NO. POS./TOTAL NO. OF ANIMALS	34/52	12/24	13/39	13/34	41/50	12/23	19/39	12/40
% OF ANIMALS WITH POS. RESULT	65	50	33	29.5	82	52.5	55	30
NO. OF EXP. GROUPS	5	2	2	3	5	2	2	3

TEXT-FIG. 2. Mean values for delayed hypersensitivity (skin infiltration) and thyroiditis in control animals and guinea pigs treated with 6MP-in different dosages (75 mg/kg and 150 mg/kg) and route of administration (intramuscular and intraperitoneal), according to schedule I.



TEXT-FIG. 3. Mean values for serum antibody titer, skin test, and thyroiditis in control animals and guinea pigs treated with 6-MP (150 mg/kg intraperitoneally) according to schedule III (early treatment).

at day 31. However the severity of thyroiditis was less strong; it was comparable to the severity of thyroiditis of control animals 21 days after immunization.

Treatment with 6-MP after development of an immune response:—The effect of 6-MP treatment which was initiated only 10 days after immunization according to schedule V resulted in a similar suppression of thryoiditis as in animals treated during the entire course of immunization. Delayed hypersensitivity was only mildly suppressed by this treatment (Text-fig. 4).

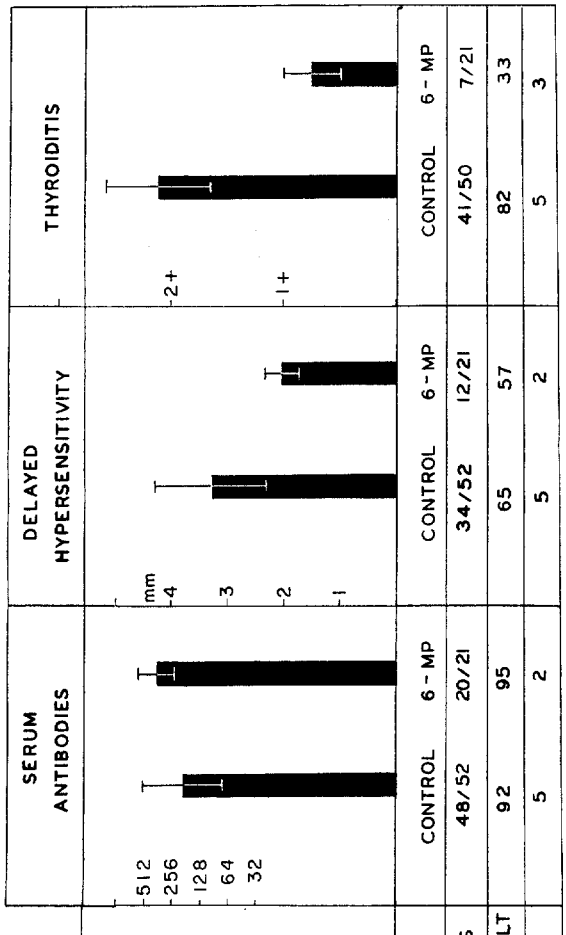
Treatment with 6-MP after development of thyroiditis.—The treatment so far described can be called prophylactic, since it was initiated before the development of thyroiditis. In the next series of experiments treatment was started after the onset of thyroiditis in order to evaluate its curative effect on immune thyroiditis.

Late treatment beginning at day 20 according to schedule VI resulted in definite depression of delayed hypersensitivity (Text-fig. 5): Only 31 per cent of the 6-MP–treated animals showed a positive delayed skin reaction in contrast to 90 per cent of the control animals. The examination of the thyroid gland of the majority of such treated animals showed marked changes in the histological pattern of immune thyroiditis. The perivascular spaces still appeared enlarged, but the round cell infiltration had disappeared (Fig. 3). The changes in the epithelial cells were still visible, although to a lesser degree in most animals.

The evaluation of thyroiditis in these animals was done according to the degree of inflammation. 6 out of 14 animals have shown no more signs of inflammation and had an almost normal appearance of the epithelial cells (Fig. 4) and 2 animals showed enlarged perivascular spaces beside swelling of the epithelial cells. These 8 animals were classified as negative. 3 animals exhibited considerable swelling of the epithelial cells as seen in Fig. 3 and scattered foci of perivascular round cell infiltrations. These animals were classified as still having mild thyroiditis. 3 animals showed no influence on the pattern of thyroiditis.

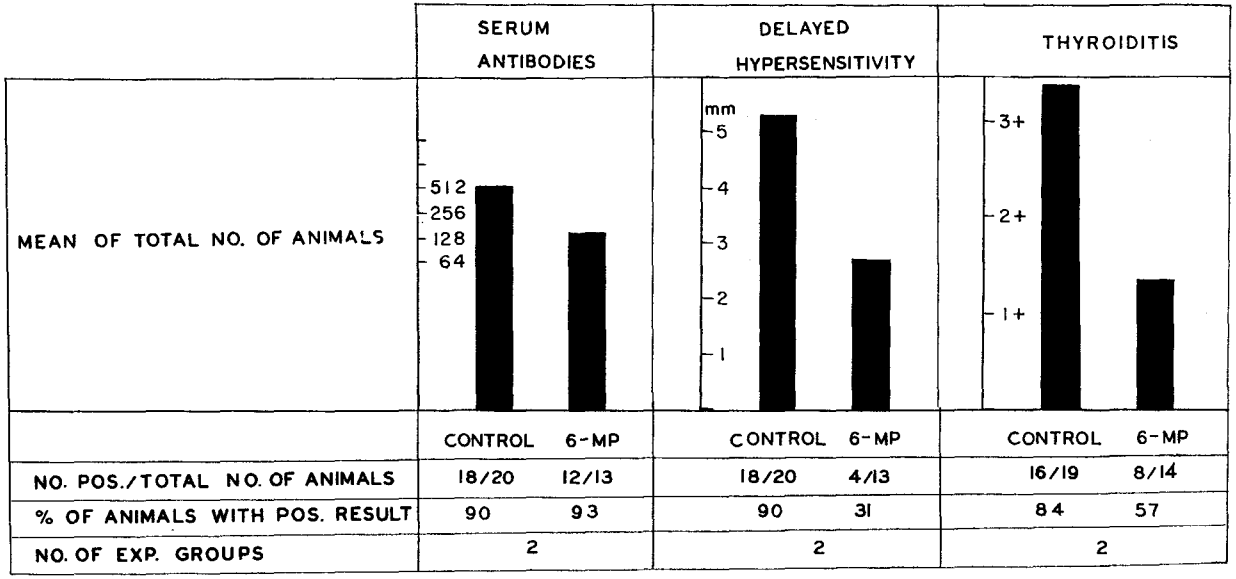
The effect of longer treatment with 6-MP initiated at day 20 according to schedule VII is summarized in Text-fig. 6. Delayed hypersensitivity was definitely depressed by this treatment, only 33 per cent of the animals showed a positive skin test. None of the 6-MP treated animals showed an immediate skin reaction of the Arthus type in contrast to 2 out of 18 control animals. In contrast to an 100 per cent incidence of severe thyroiditis in the control animals, only 4 treated guinea pigs had a comparable degree of thyroiditis. In 12 out of the 20 treated animals, a marked diminution of round cell infiltration was observed, however they still showed swelling of the epithelium and some scattered foci of infiltration (result recorded weak positive). 4 animals had an entirely negative appearance of the thyroid gland.

Effect of 6-MP using large amounts of antigen for immunization.—One group of 10 animals was immunized with 10-fold the usual amount of antigen. This immunization resulted in a weaker appearance of the immune response than in the animals immunized with the small amount of thyroid extract (Text-fig. 7).

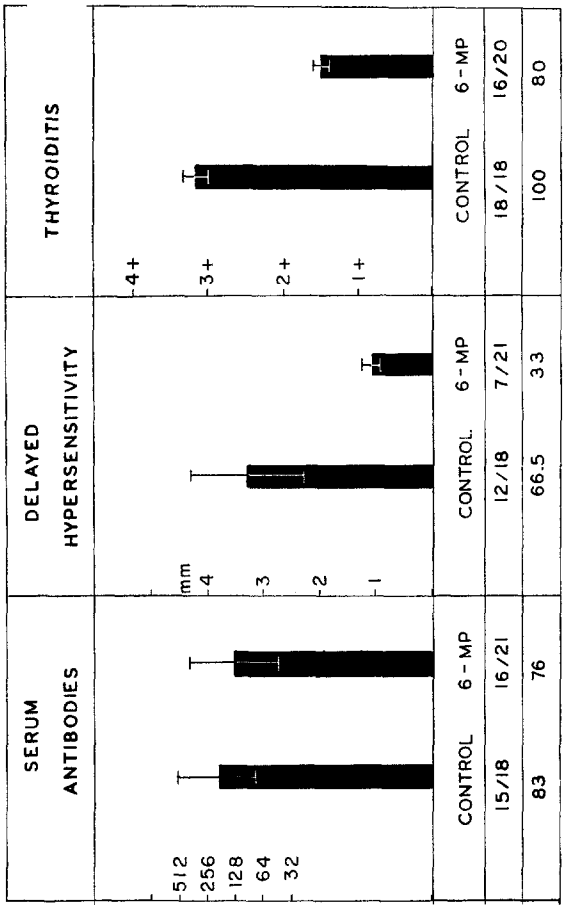


	CONTROL	6-MP	CONTROL	6-MP	CONTROL	6-MP
NO. POS./TOTAL NO. OF ANIMALS	48/52	20/21	34/52	12/21	41/50	7/21
% OF ANIMALS WITH POS. RESULT	92	95	65	57	82	33
NO. OF EXP. GROUPS	5	2	5	2	5	3

TEXT-FIG. 4. Mean values for serum antibody titer, skin test, and thyroiditis in control animals and guinea pigs treated with 6-MP (150 mg/kg intraperitonally) according to schedule V (late treatment).



TEXT-FIG. 5. Mean values for serum antibody titer, skin test, and thyroiditis in control animals and guinea pigs treated with 6-MP (150 mg/kg intraperitoneally) according to schedule VI (treatment after thyroiditis had developed).



TEXT-FIG. 6. Mean values for serum antibodies, skin test, and thyroiditis in control animals and guinea pigs treated with 6-MP (150 mg/kg intraperitoneally) according to schedule VII (treatment after thyroiditis had developed).

Treatment with 6-MP (150 mg/kg), according to schedule I, of 11 animals immunized with this large quantitity of thyroid extract showed a positive skin reaction in the same proportion as in the control animals. However none of these animals developed thyroiditis. (Text-fig. 7).

Immune Response and Incidence of Thyroiditis in Animals Treated with Aminopterin.—Treatment schedules I, III to V were used in a total of 67 guinea pigs.

Treatment according to schedule I.—The effect of aminopterin given in a dose of 5 mg/kg intraperitoneally, according to schedule I, is shown in Text-fig. 8. This treatment lead to a marked depression of antibody formation; only 2 out of 28 animals showed demonstrable serum antibodies. The incidence of delayed hypersensitivity and thyroiditis was also significantly depressed, although not as strikingly as the serum antibody formation.

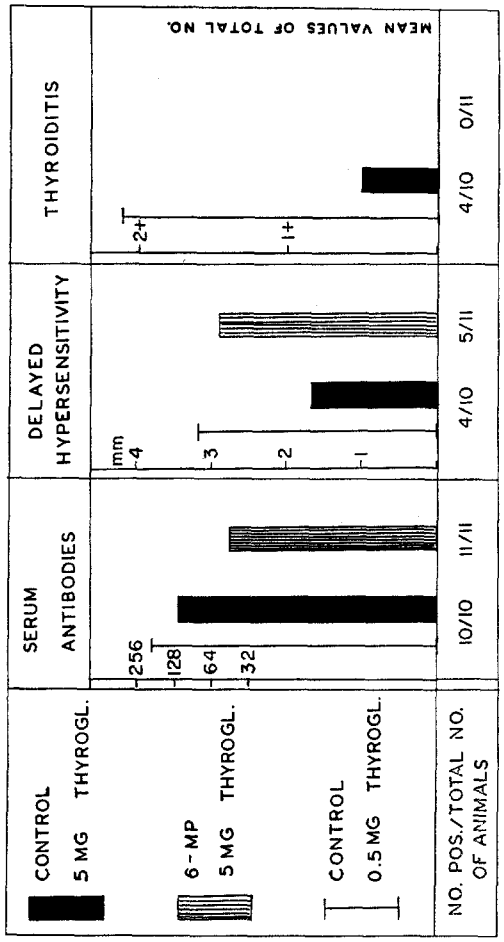
No qualitative changes in the histological pattern of thyroiditis were found. The 2 animals which showed serum antibodies did not exhibit thyroiditis. Among the treated animals with a positive skin test 40 per cent exhibited thyroiditis.

Treatment in the early phase of immunization.—The effect of aminopterin (5 mg/kg) administered for the first 10 days according to schedule III is shown in Text-fig. 9. Antibody formation was markedly depressed by this treatment, however to a lesser extent than in animals treated during the entire period of the experiment. Delayed hypersensitivity was only mildly influenced. Thyroiditis was equally well suppressed as with the treatment during the whole course of immunization.

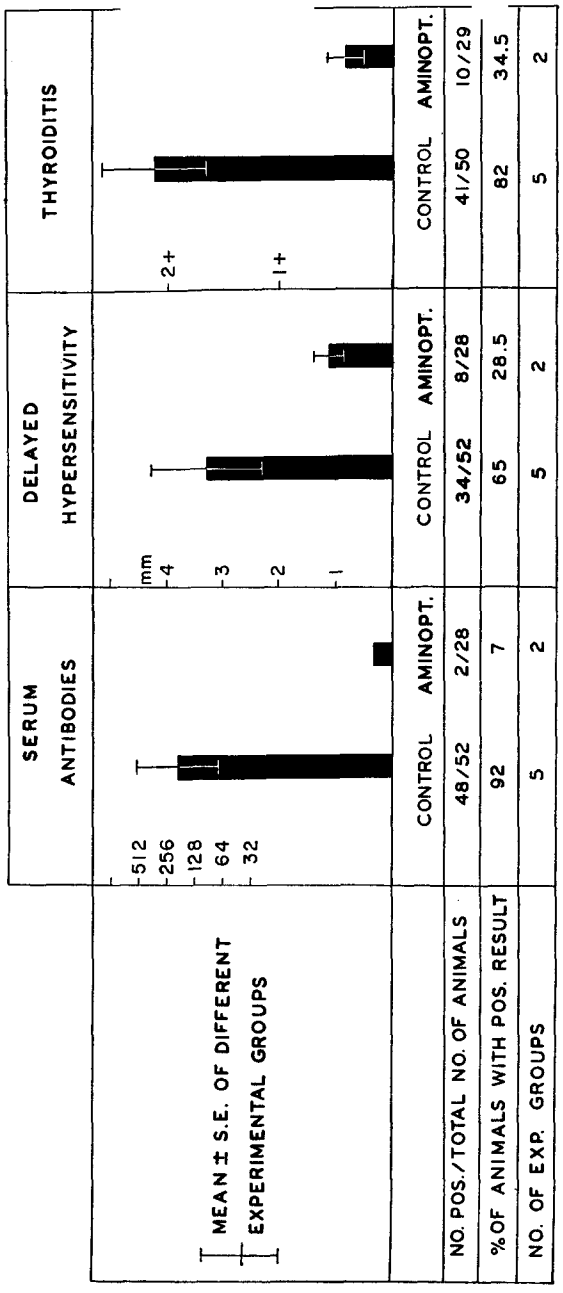
Treatment with aminopterin after development of an immune response.—Late treatment in the second period of immunization (5 mg/kg), according to schedule V, did not influence immune response and thyroiditis (Text-fig. 10). However when the double dose of aminopterin was given (10 mg/kg), according to schedule V, immune response as well as thyroiditis were depressed (Text-fig. 10).

Effect of 6-MP and Aminopterin on the Number of Circulating Leucoytes.—Examination of the leucocytes at different time intervals resulted in the following findings. A rise of the number of polymorphonuclear leucocytes was found in the control animals from 3460/mm^3 at day 0 to 9100/mm^3 day 20 (average values of 40 animals). The number of mononuclear cells however stayed constant during the entire period (average values of 40 animals 3050/mm^3 at day 0 and 3250/mm^3 at day 21).

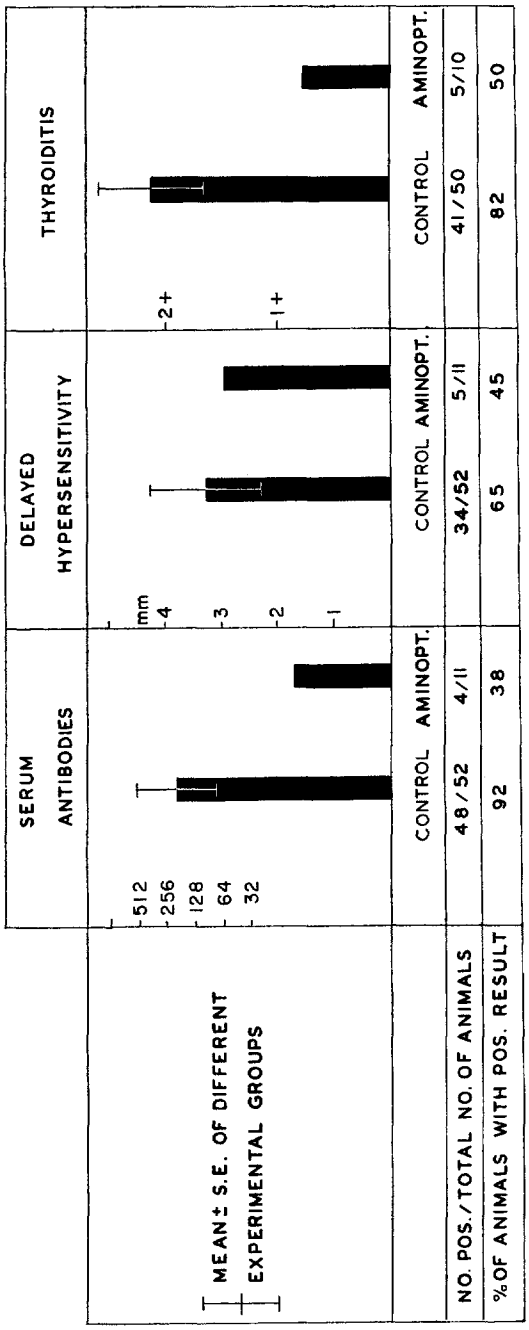
The number of polymorphonuclear leucocytes was strongly depressed by 6-MP treatment. No increase as seen in the control animals occurred in the animals treated with 150 mg/kg 6-MP (average values of 21 animals at day 10, 2600/mm^3 and at day 20, 1620/mm^3.) However after discontinuation of



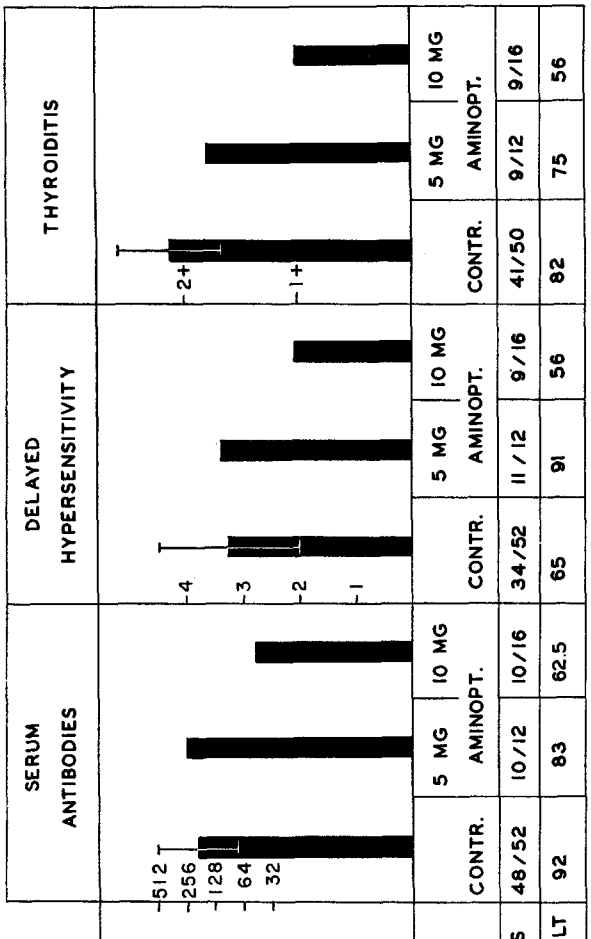
TEXT-FIG. 7. Mean values for serum antibody titer, skin test, and thyroiditis of control animals and guinea pigs immunized with 10-fold the usual amount of thyroglobulin (thyrogl.) and such immunized guinea pigs treated with 6-MP (150 mg/kg intraperitoneally) according to schedule I.



TEXT-FIG. 8. Mean values of serum antibody titer, skin test, and thyroiditis in control animals and guinea pigs treated with aminopterin (aminopt.) (5 mg/kg intraperitoneally) according to schedule I.



TEXT-FIG. 9. Mean values of serum antibody titer, skin test, and thyroiditis in control animals and guinea pigs treated with aminopterin (2 mg/kg intraperitoneally) according to schedule III (early treatment).



	SERUM ANTIBODIES			DELAYED HYPERSENSITIVITY			THYROIDITIS		
	CONTR.	5 MG AMINOPT.	10 MG AMINOPT.	CONTR.	5 MG AMINOPT.	10 MG AMINOPT.	CONTR.	5 MG AMINOPT.	10 MG AMINOPT.
NO. POS./TOTAL NO. OF ANIMALS	48/52	10/12	10/16	34/52	11/12	9/16	41/50	9/12	9/16
% OF ANIMALS WITH POS. RESULT	92	83	62.5	65	91	56	82	75	56

TEXT-FIG. 10. Mean values of serum antibody titer, skin test, and thyroiditis in control animals and guinea pigs treated with aminopterin (5 and 10 mg/kg intraperitoneally) according to schedule V (late treatment).

treatment (schedule III) the number of leucocytes increased rapidly, and no more depression of the polymorphonuclear leucocytes was found at the end of the experiment. The number of mononuclear cells was not significantly depressed by 6-MP treatment (average values of 21 animals: 3200/mm^3 at day 0 and 2950/mm^3 at day 21).

Treatment with aminopterin resulted in similar findings, however the depression of the number of polymorphonuclear leucocytes was not as marked as with 6-MP (average value of 29 animals: 2900/mm^3 at day 0 and 5500/mm^3 at day 21). Again no influence on the number of mononuclear cells was observed.

No correlation was found in a total of 50 animals treated with 6-MP or aminopterin between the number of polymorphonuclear leucocytes, mononuclear cells, and degree of thyroiditis.

DISCUSSION

The reported experiments have demonstrated that two different antimetabolites, the antipurine antagonist 6-mercaptopurine, and the antifolic acid antagonist aminopterin, suppress the development of immune thyroiditis in guinea pigs. Both compounds depress in a similiar manner delayed hypersensitivity to thyroglobulin in these animals, whereas serum antibody formation is only suppressed by aminopterin.

The results will be discussed from two different points of view: First, the pharmacological suppression of immune thyroiditis serves as a tool for the investigation of the pathogenesis of this disease. Second, the mechanism by which these compounds suppress immune thyroiditis will be analyzed.

The pathogenesis of experimental immune thyroiditis is still unknown. Although a number of facts strongly suggest delayed hypersensitivity to be of major importance (18–21) some data also indicated serum antibodies as a possible trigger factor (22).

The fact that the two antimetabolites have a different effect on antibody formation is important in the evaluation of immune thyroiditis. If antibodies would determine the onset of thyroiditis, aminopterin should have suppressed thyroiditis to a much greater extent than 6-MP which was ineffective on antibody formation. However, the suppressive action on thyroiditis of the two compounds was very similiar, and the experimental disease occurred in aminopterin-treated animals in which antibody formation was not demonstrable. These data strongly speak against a major participation of circulating antibodies in the pathogenesis of this disease.

There was a close relationship between animals showing a positive skin test and thyroiditis confirming previous observations (18, 19). Under certain conditions the antimetabolites suppressed thyroiditis without influencing the result of the skin test. In animals treated only during the first 10 days, this result on day 20 can be explained by a delay in the immune response as well

as in the onset of thyroiditis. Thus, these animals would be comparable to non-treated animals at day 10, where delayed hypersensitivity but not yet thyroiditis is found. Indeed, the animals treated for 10 days later develop thyroiditis. In other experiments the different effect on delayed type of hypersensitivity and immune thyroiditis may have another reason relevant to the question of the mode of action by which thyroiditis is suppressed.

It has been proposed that prevention of experimental immune disorders by antimetabolites is mediated by the suppression of immune response (12). In this connection we will confine our discussion only to delayed type of immune response since the participation of serum antibodies detected in this study could be eliminated as a trigger mechanism of immune thyroiditis.

In a number of experimental conditions, thyroiditis was depressed without influencing similiarly delayed hypersensitivity, which seem to contradict the above mentioned assumption. Thus only a very mild suppressive effect on delayed hypersensitivity was observed in the animals treated from day 10 to day 20 of the experiment, while thyroiditis was markedly depressed by this procedure. Furthermore the effect on immune response and thyroiditis did not show the same dependence of the route of administration. While 6-MP given intramuscularly has a stronger effect on the suppression of delayed hypersensitivity than intraperitoneal administration, no difference was found with respect to the effect on thyroiditis. Finally, higher doses of 6-MP were necessary to depress delayed hypersensitivity than thyroiditis.

These data must be taken into account in the evaluation of the mechanism by which these antimetabolites suppress thyroiditis. Two possible modes of action may be considered, not involving the mediation of the suppression of the immune response: (*a*) Suppression of inflammatory cells. (*b*) The anti-inflammatory vascular action (independent of antimitotic activity).

Immune thyroiditis involves almost exclusively mononuclear inflammatory cells. In animals exhibiting thyroiditis, these cells disappear rather promptly upon 6-MP treatment. If this disappearance would be the result of the antimitotic action, a generalized diminution of these cell elements should be observed. However, the mononuclear cells in the peripheral circulation were not significantly reduced by this treatment. The only cell type which was suppressed was the polymorphonuclear leucocyte, which seems not to participate in immune thyroiditis.

Page *et al.* first suggested an anti-inflammatory activity of 6-MP (23). This action would especially affect the invasion of mononuclear cells into a locus of inflammation. These findings are based on the egg white–skin reaction, which may not be an ideal test for investigating non-specific inflammation and which may be closely related to immune phenomena. The anti-inflammatory effect observed with 6-MP in the egg white test lasted only 48 hours after discontinuation of medication.

In this connection it is worth noting that the inflammation at the site of the injection of Freund adjuvant was not influenced by the anti-metabolite treatment, macroscopically as well as microscopically (24). Furthermore, the most potent anti-inflammatory compounds such as hydrocortisone (15 mg/kg/day), dexamethasone (15 mg/kg/day), prednisolone (15 mg/kg/day), and phenylbutazone (100 mg/kg/day) did not influence delayed hypersensitivity, antibody formation as well as thyroiditis (24).

The ineffectiveness of anti-inflammatory compounds on immune thyroiditis in guinea pigs and the short duration of the anti-inflammatory effect of 6-MP in the egg white test speaks against the possibility that thyroiditis is inhibited on such a basis. It is obvious that such short lasting anti-inflammatory activity would not prevent immune thyroiditis if the treatment is confined to the first 10 days of immunization, in which period there is no inflammation in the thyroid gland.

The reported experiments do not allow a final conclusion on the mechanism of suppression of the immune thyroiditis by 6-MP and aminopterin. Although there is no complete relation between the effect on the immune response and thyroiditis, it seems probable that the action on thyroiditis is related to the action on the changes of the immune response.

The fact that 6-MP is still effective on thyroiditis once it has developed may be of practical importance for the use of this drug in human patients. Whereas one purpose for its clinical application is the suppression of immune response in homotransplantation, another purpose is the treatment of a disease in which immune phenomena play a major role. Our results support such a possibility for the treatment of diseases, such as immune hemolytic anemias and systemic lupus erythematosus.

SUMMARY

The influence of 6-mercaptopurine and aminopterin was studied on immune response and immune thyroiditis in guinea pigs immunized with thyroid extract.

1. Both compounds depressed delayed hypersensitivity to thyroglobulin and immune thyroiditis.

2. Antibody formation to thyroglobulin was strongly depressed by aminopterin but not significantly influenced by 6-mercaptopurine.

3. Immune response and thyroiditis were suppressed as long as the compounds were administered; after discontinuation of treatment, immune response and thyroiditis appeared in the same time intervals as observed in control animals after initiation of immunization.

4. Treatment with 6-mercaptopurine and aminopterin 10 days after immunization lead to suppression of delayed hypersensitivity and thyroiditis.

5. Treatment with 6-mercaptopurine of animals after onset of thyroiditis lead to suppression of delayed hypersensitivity and disappearance or diminution of round cell infiltration in the thyroid.

6. The results are discussed in terms of the pathogenesis of experimental immune thyroiditis, the mode of action of these antimetabolites on this experimental immune disease, and in view of the potential value of these compounds in human diseases.

The authors wish to acknowledge the valuable technical assistance of Miss Marthe Fauconnet and Miss Cecile Zuber.

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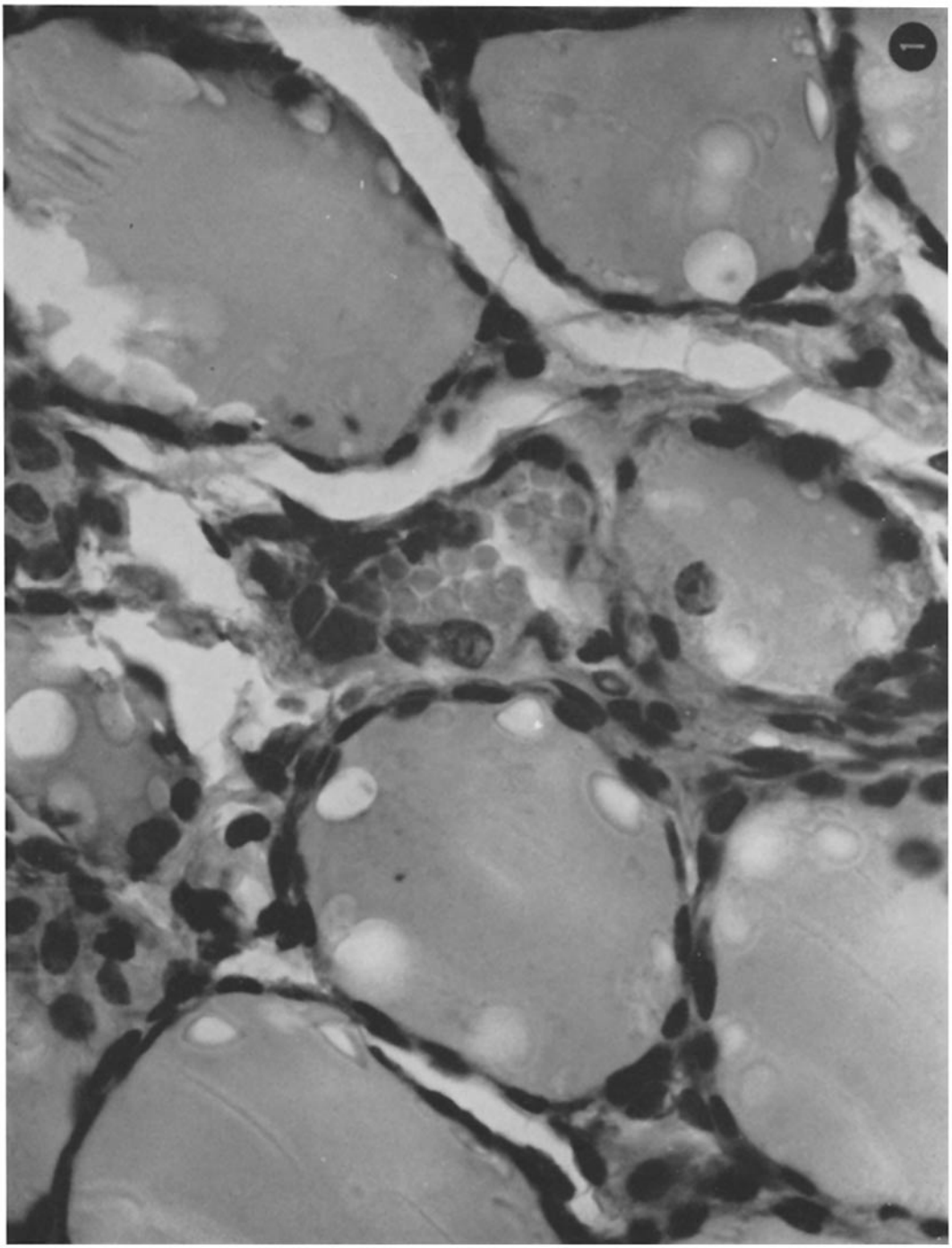
EXPLANATION OF PLATES

PLATE 81

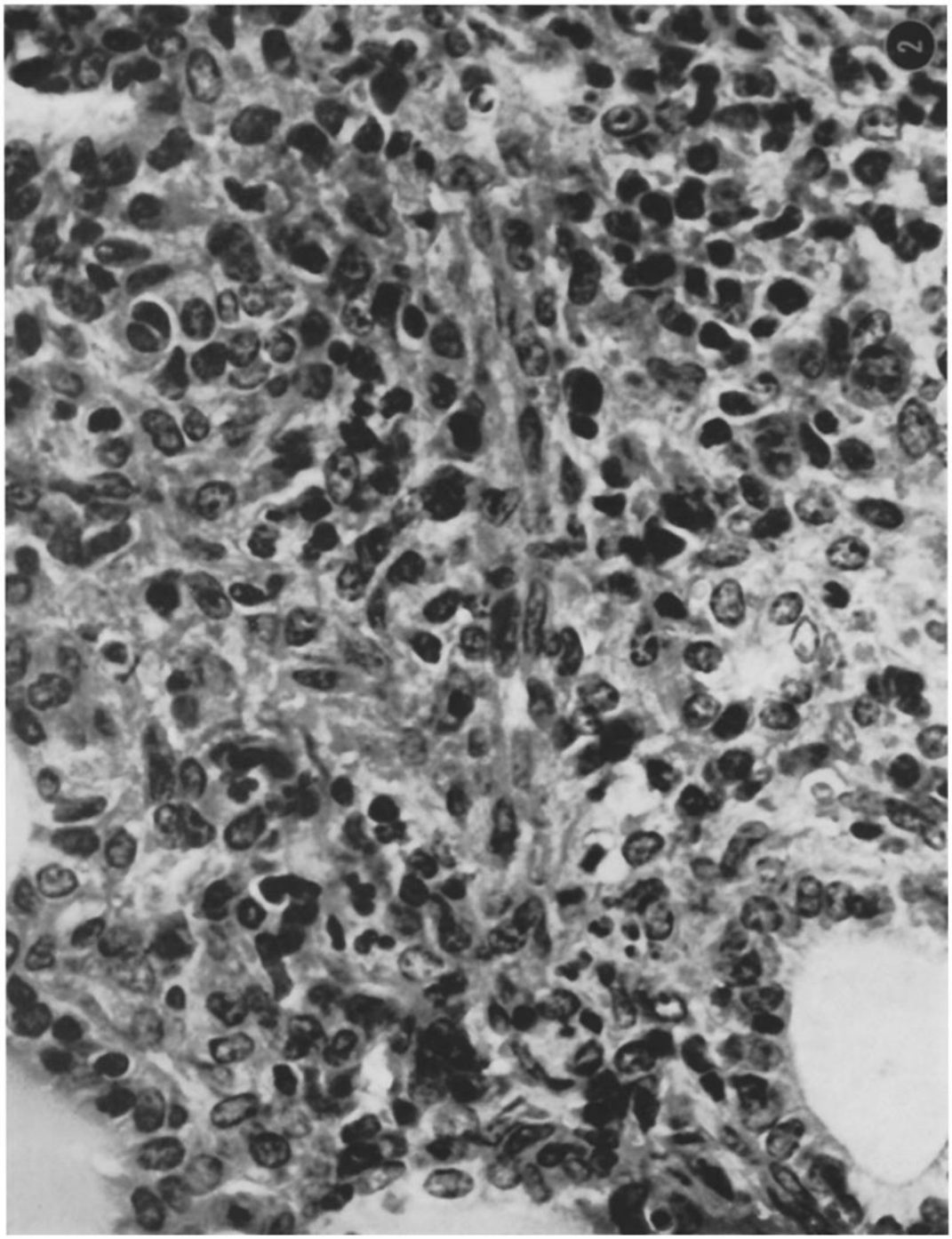
FIG. 1. Structure of the normal guinea pig thyroid gland. Note the flattened shape of the epithelial cells in the cross section, and the blood vessel in the center with a small perivascular space. ×500.



Spiegelberg and Miescher: Experimental immune thyroiditis)

PLATE 82

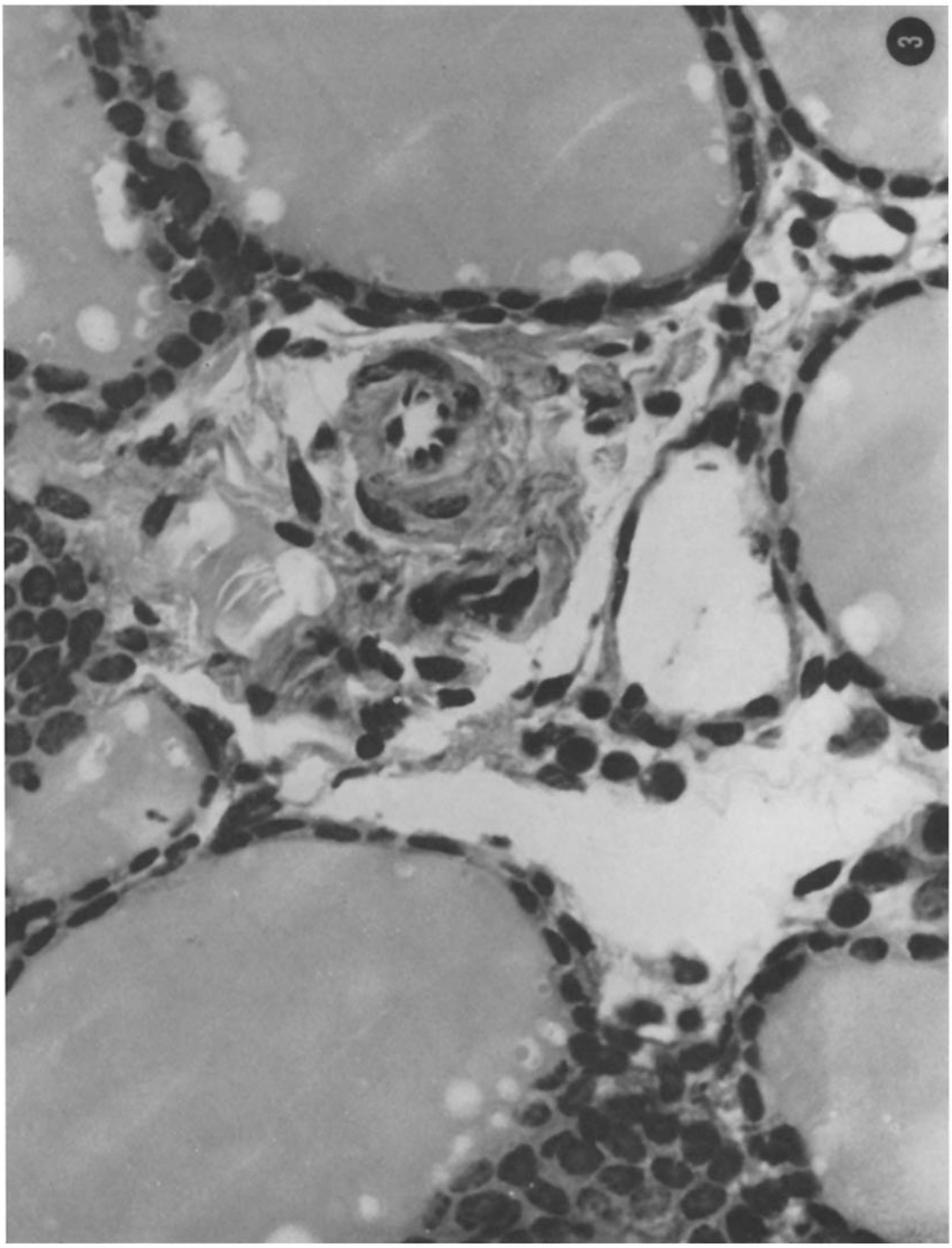
FIG. 2. Immune thyroiditis in guinea pigs. The epithelial cells are swollen and appear cuboidal. The perivascular spaces are infiltrated with histiocytes and lymphocytes. Note compressed blood vessel in center. ×500.



(Spiegelberg and Miescher: Experimental immune thyroiditis)

PLATE 83

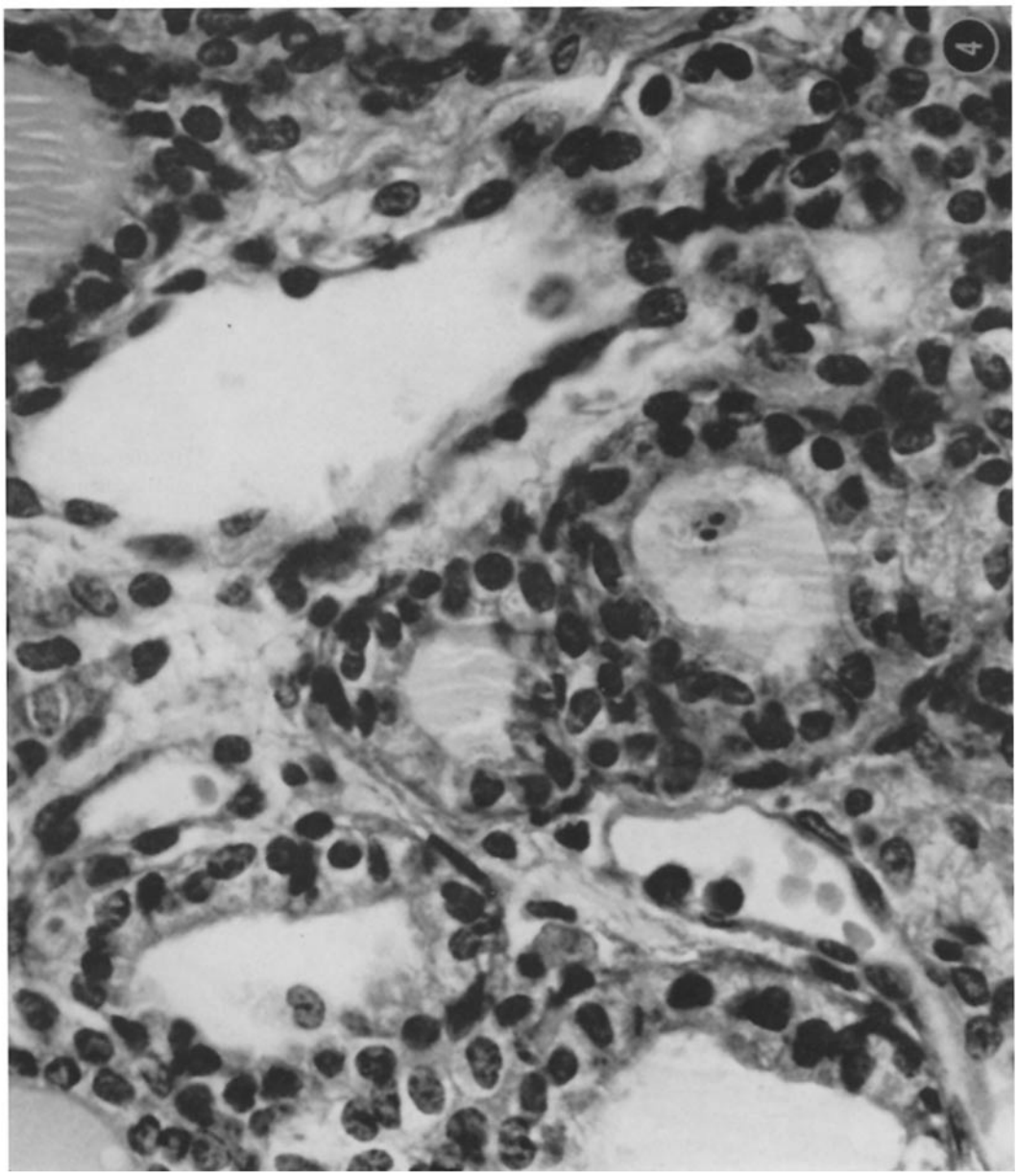
FIG. 3. Thyroid gland in animals treated with 6-MP after development of thyroiditis (schedules VI and VII). The perivascular spaces are still enlarged but free of inflammatory cells. Note enlarged perivascular space and light swelling of endothelial cells of blood cells. ×500.



(Spiegelberg and Miescher: Experimental immune thyroiditis)

PLATE 84

FIG. 4. Appearance of the thyroid gland in animals treated with 6-MP after development of thyroiditis (treatment schedule VI and VII) with only mild swollen thyroid cells and absence of perivascular round cell infiltration. ×500.



(Spiegelberg and Miescher: Experimental immune thyroiditis)