tary TSH(2) have not been confirmed(13, 14). However, the capacity of antisera to bovine TSH to neutralize human TSH may not be an all or none reaction but may be dependent upon quantitative relationships between antigen and antibody. This has been suggested by the reports of Werner and Tierney(15) and of Levy, McGuire and Heideman(16). This important relationship now being investigated is particularly germane to development of immunoassay methods to measure human TSH.

Summary. A modification of the Querido bio-assay for thyrotropin is presented along with log dose-response curves for USP Reference Standard Thyrotropin in 2 different strains of mice. The immunobiologic effects of antisera to bovine thyrotropin (TSH) on either endogenous or exogenous TSH were determined in guinea pigs, intact or hypophysectomized rats and mice. In thyroxinized guinea pigs and thyroxinized intact or hypophysectomized rats, passively administered rabbit antiserum to bovine TSH neutralized the biologic activity of exogenous bovine TSH. Rabbit antiserum passively administered to guinea pigs, rats or mice inhibited the effects of propylthiouracil (PTU) on goitrogenesis and on radioactive iodine uptake, indicating biologic neutralization of endogenous TSH in these species. These findings suggest a molecular similarity of guinea pig, rat and mouse thyrotropin.

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Inhibition of Established Tuberculin Hypersensitivity by Methotrexate. (29282)

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The induction of immune responses in various animal species is inhibited by the antifol methotrexate (aminomethylpteroylglutamic acid(1,2). This drug-induced depression of immune responses has been most thoroughly investigated in guinea pigs in which doses of methotrexate which caused no obvious systemic toxicity suppressed the development of delayed hypersensitivity, antibody production, and the specific febrile response to purified protein antigens(2). Methotrexate treatment has also inhibited the development of experimental allergic encephalomyelitis(3), autoimmune thyroiditis(4), and the homograft response in guinea pigs(5).

Preliminary studies of the mechanism of action of methotrexate on the immune responses of guinea pigs suggested that the drug

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TABLE I. Suppression of Actively Established Tuberculin Hypersensitivity in Strain 2 Guinea Pigs by Methotrexate.

	No. of days after inoculation of heat-killed B.C.G.					
	14	15	30	40	41	50
Size of skin reaction (mm)	18 × 15	Methotrexate started	Methotrexate-treated: 10 × 10	0	Methotrexate stopped	21 × 20
	17 × 15		0	0		20 × 19
	15 × 14		0	0		20 × 17
	15 × 14		0			20 × 19
	15 × 13		Controls: 21 × 19	20 × 20		20 × 20
	15 × 12		20 × 20	20 × 19		20 × 18
			20 × 18	20 × 17		
			20 × 18			

selectively inhibited the proliferation of immunologically active cells(6). These results suggested in turn that prolonged treatment with methotrexate might well depress an established immune response by inhibiting further multiplication of immunologically active cells. Because the suppression of an established immune reaction might be useful in treatment of human diseases thought to be due to an autoimmune response, the effect of the drug on well-established tuberculin hypersensitivity was investigated, since delayed hypersensitivity, of which the tuberculin response is the classical model, is considered by many to represent the immune mechanism involved in autoimmune diseases.

The results indicate that inhibition of established tuberculin hypersensitivity occurs within a surprisingly short period in inbred guinea pigs treated with methotrexate.

Materials and methods. Highly inbred, histocompatible Strain 2 and Hartley albino guinea pigs of both sexes weighing between 300 and 450 g were employed. Animals were inoculated with 0.5 mg of a lyophilized and autoclaved preparation of B.C.G. culture pellicles obtained from Dr. S. Chaparas of N.I.H. and suspended in 0.5 ml of equal parts Freund's incomplete adjuvant (80% arlacel A, 20% Bayol F) and saline, 0.05 to 0.1 ml being inoculated into 7-10 sites in the nuchal area. A daily dose of 5 mg of methotrexate (purchased from Lederle Laboratories, Pearl River, N.Y.) dissolved in 1 ml of saline was administered intraperitoneally. Animals were skin tested intradermally with 0.1 ml containing 0.005 mg of P.P.D. (purchased from Merck, Sharp & Dohme, West Point, Pa.). Reactions were read at 6, 24 and 48 hours after P.P.D. inoculation but only slight reactions were seen at 6 hours. Reactions were maximal at 18-24 hours. Those over 10 mm in diameter were considered significant. Smaller reactions were read as negative. Reactions are reported as the largest diameter of redness and induration and the diameter perpendicular to this 20 hours after skin test. Animals were skin tested twice only.

Lymph-node cell suspensions were made by a previously described method(6). The intraperitoneally inoculated suspensions of lymph node cells from B.C.G. sensitized strain 2 guinea pigs contained 3 to 4 × 10^8 cells, a donor-to-recipient ratio of approximately 1.2 to 1. Whole-body x-irradiation of 300 r was administered by Mr. Henry Meyer, Radiation Branch, N.I.H. over 2.43 minutes from a 250 kv source using simultaneous dorsal and ventral portals.

Results. After preliminary experiments with results similar to those reported here, 14 Strain 2 guinea pigs were inoculated with heat-killed B.C.G. suspended in Freund's incomplete adjuvant. After 14 days, 6 animals were selected at random and skin tested with P.P.D. The tuberculin test was positive in all animals (Table I), as noted in previously reported experiments employing similar con-

ditions(6). On the 15th day after B.C.G. inoculation the animals were divided into 2 groups of 7 each. One group received 5 mg of methotrexate daily, the other, saline inoculations. After 15 days of this treatment, 30 days after inoculation of B.C.G., 4 animals from each group were again tested with P.P.D. The animals receiving saline had larger reactions than those previously noted on the 14th day after sensitization, while of those receiving methotrexate 3 had completely negative skin tests and one had a minimally positive reaction of 10 × 10 mm (Table I). One animal receiving methotrexate and tested on the 30th day was found dead on the 32nd day after B.C.G. inoculation, but autopsy revealed only a non-specific pneumonitis. This was the only animal to die during or following methotrexate treatment during several experiments employing the drug.

The methotrexate treatment was continued in all animals until the 40th day after B.C.G. inoculation, after 25 days of methotrexate treatment. The 3 animals in each group which had not been skin tested on the 30th day after B.C.G. inoculation were then skin tested with P.P.D. Results were similar to the findings of 10 days previously (Table I).

Methotrexate treatment was stopped and 10 days later, 50 days after inoculation of B.C.G., the animals which had been skin tested on the 40th day after B.C.G. inoculation were again tested. All of the animals in both groups had positive skin reactions to P.P.D. (Table I).

Similar results have been obtained in Hartley guinea pigs (Table II) except that the tuberculin response was more difficult to suppress completely. Actively established tuberculin hypersensitivity was, therefore, depressed by methotrexate in both strains of guinea pig.

During the above studies, all of the experimental animals were noted to have formed deep skin ulcers at the nuchal sites of inoculation of adjuvant and heat killed B.C.G. within 2 weeks of their inoculation. Such ulcerations occurred at the site of inoculation of heat killed B.C.G. suspended in either adjuvant or saline, but not at the site of adjuvant inoculation alone. The first appearance

TABLE II. Depression of Actively Established Tuberculin Hypersensitivity in Hartley Guinea Pigs by Methotrexate.

		No. of days after inoculation of heat killed B.C.G.		
		16	17	40
Size of skin reaction (mm)	Methotrexate started	21 × 17	15 × 12	13 × 13
	Methotrexate-treated	22 × 20	12 × 12	15 × 12
		20 × 20	12 × 11	0
		25 × 22	10 × 10	0
	Controls	18 × 14	25 × 23	30 × 25
		18 × 18	21 × 20	28 × 25
		23 × 20	24 × 20	28 × 25
		20 × 22	27 × 22	26 × 25

and subsequent size of the skin ulcers seem to be directly related respectively to the time of first appearance of tuberculin hypersensitivity and to the intensity of the sensitivity developed(7). These findings are similar to those of Clawson(8) who showed that the development of skin ulcers and other necrotic lesions under similar conditions depended on the presence of tuberculin hypersensitivity.

During the course of methotrexate treatment of both Strain 2 and Hartley guinea pigs the ulcers in the nuchal area were first noted to decrease in size, and, by 21 days after methotrexate was started, they had completely closed. Histologic examination of the former ulcer sites at this time showed epithelial and hair follicle regeneration. A slight dermal fibrosis and a moderate chronic dermatitis were present. The ulcerations in control animals increased in size throughout the studies reaching finally from 15 to 20 mm in diameter.

Because of the prolonged treatment with a toxic agent undergone by the test animals, weight changes, hematograms, and reaction to a non-specific inflammatory agent were measured in control animals and in animals which had just finished 25 days of methotrexate treatment. The average result of tests on the Strain 2 guinea pigs employed in the above experiment are reported in Table III. Both groups of animals gained weight. The methotrexate-treated group became anemic,

TABLE III. Hematologic and Weight Changes in Tuberculin-Sensitized Control Animals and in Animals Receiving Methotrexate for 25 Days.

	Wt change (g)	Hemoglobin (g %)	White blood cell count (per cu mm)	Differential white blood cell count: Polymorphonuclear leukocytes	Differential white blood cell count: Lymphocytes	Circulating lymphocytes (per cu mm)
Controls	+86*	16.0	7500	38%	62%	4650
Methotrexate treated	+98	10.0	9500	66%	34%	3230

* All results represent average of findings in at least 4 Strain 2 guinea pigs.

but white cell counts were similar in both groups. Of interest in the methotrexate-treated group was a reversal in the normal differential white blood cell counts seen in control animals (38% polymorphonuclear leukocytes and 62% lymphocytes). In the methotrexate-treated animals the counts were 66% polymorphonuclear leukocytes and 34% lymphocytes.

Since inhibition of delayed hypersensitive responses had been reported in animals with reduced circulating lymphocyte counts(9,10), animals with well established tuberculin hypersensitivity were exposed to 300 r total body X-irradiation. After 3 days, when white blood cell counts averaged 1200 cells cu mm, with circulating lymphocyte counts of 840, the animals were again tuberculin tested. No diminution in the size, and only a questionable decrease in intensity of the reactions were observed, confirming previously published findings(11). In the methotrexate-treated animals the suppression of tuberculin hypersensitivity was probably not due to the moderate decrease noted in the circulating lymphocyte count.

In addition (not shown in Table III) the group of B.C.G. tuberculin-inoculated animals which had been treated with 5 mg a day of methotrexate for 25 days were given an intradermal inoculation of turpentine. The size and intensity of the resulting inflammation after 24 hours did not differ significantly from the response of tuberculin-inoculated animals which had not received methotrexate. The drug did not seem, therefore, to inhibit a non-specific inflammatory response.

In the above reported experiments the tuberculin hypersensitivity had been established by active sensitization. Since the persistence of heat-killed B.C.G. in the actively sensitized animals may have altered their immune responses, an experiment was carried out on Strain 2 guinea pigs in which tuberculin hypersensitivity was established by transfer of isologous lymph node cells from tuberculin positive donors (adoptive immunity). Fourteen days after such a cell transfer to 4 recipient animals, all recipients had positive tuberculin skin tests (Table IV) as in previously reported, similarly conducted experiments(6).

At this point daily methotrexate treatment was started and continued for 14 days on 2 of the 4 animals. After this treatment, 28 days after the cell transfer, all 4 animals were again tuberculin tested. As shown in Table IV, the controls which had not received methotrexate remained tuberculin positive while the methotrexate-treated animals were completely negative.

Discussion. The results of these studies indicate that both actively and passively established tuberculin hypersensitivity may be suppressed in Strain 2 guinea pigs by treatment with methotrexate. In actively sensitized animals the tuberculin sensitivity recurs within 10 days of cessation of methotrexate treatment. The suppression of the tuberculin hypersensitivity does not seem due to a depression of circulating lymphocytes or

TABLE IV. Suppression of Passively Established Tuberculin Hypersensitivity by Methotrexate.

	No. of days after inoculation of heat-killed B.C.G.			
	14	15		28
Size of skin reaction in mm	16 × 14	Methotrexate started	Methotrexate treated	0
	15 × 15			0
	15 × 15		Controls	15 × 15
	14 × 13			14 × 12

to a non-specific anti-inflammatory action.

The suppression of established delayed hypersensitivity by methotrexate may provide a theoretical basis for the use of drugs which suppress immune responses in treatment of established diseases thought to be autoimmune in nature, since these disease states may be related to the delayed hypersensitive response(12). Methotrexate has already been successfully employed to inhibit established experimental allergic encephalomyelitis(3) and auto-immune thyroiditis(4), both experimental diseases often considered to be autoimmune in nature, and to be related to delayed hypersensitivity.

Since the effect of methotrexate appears to be on cell multiplication rather than to be a direct cytotoxic phenomenon(6,13), the observation that delayed hypersensitivity may be suppressed after treatment with methotrexate for as short a period as 15 days strongly suggests that the life span of the effector cells involved in the tuberculin response in guinea pigs must be considerably shorter than this period. Methotrexate has no effect on passively established, but non-adoptive delayed hypersensitivity(14), possibly because no cell multiplication is necessary for expression of this reactivity. The recurrence of tuberculin hypersensitivity in actively sensitized guinea pigs after cessation of methotrexate administration could have been due to the persistence (during methotrexate administration) of cells which had been sensitized to tuberculin, but which had been prevented from multiplying by methotrexate, or, alternatively, to resensitization of the animal by the heat-killed B.C.G. still present.

Summary. Actively or passively established tuberculin hypersensitivity in Strain 2 guinea pigs is suppressed by methotrexate treatment for as short a period as 15 days. The suppression of this immune response does not seem to be due to a lymphopenic or anti-inflammatory effect of the drug.

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Cutaneous Anaphylactic Reactions with Antibodies of Different Qualities.*† (29283)

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An abundance of data exists concerning the quantity of antibody required to elicit various immunologic reactions, but considerably less is known about the various qualities of antibodies needed for these purposes. Antibody taken from hyperimmunized animals has been used almost exclusively in studies

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