EXPERIMENTAL THYROIDITIS IN RABBITS, GUINEA PIGS AND DOGS, FOLLOWING IMMUNIZATION WITH THYROID EXTRACTS OF THEIR OWN AND OF HETEROLOGOUS SPECIES

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For the past 6 years, the effects of active immunization of experimental animals with thyroid extract have been investigated in our laboratories. Extracts have been procured from homologous and heterologous species. A method for preparing comparatively undenatured and purified tissue extracts was developed.[1] Various immunologic procedures were tested in order to demonstrate thyroid-specific antibodies in the blood of immunized animals.[2,3] In the course of these studies the antigenic nature of crude saline extract of rabbit thyroid was clearly recognized in rabbits. Rabbit thyroid antibodies were demonstrated in the circulating blood following intradermal injection of minute amounts (0.05 ml.) of thyroid extract combined with the complete Freund adjuvants. These antibodies were organ-specific and reacted with rabbit thyroid extract. It was found that thyroidectomized rabbits, injected with autologous thyroid extract, produced antibodies which reacted with the autologous extract as well as with thyroid extracts obtained from other rabbits. The antigen content in the thyroid of immunized rabbits was considerably reduced in comparison to that procurable from the glands of nonimmunized animals. This reduction seemed to correlate with the titer of circulating antibody in the immunized animal.[4] In the thyroids of the immunized rabbits, histologic alterations were observed concerning which a brief preliminary description was given.[5] The resemblance of the histologic pattern in experimental thyroiditis to that of chronic thyroiditis in man has been discussed recently.[6,7]

It is the purpose of this paper to provide a detailed and inclusive analysis of the combined data obtained from investigations in the rabbit, dog, and guinea pig, carried out during the past 6 years. The relationship between auto-antibody titer and alterations in the thyroid gland suggested a direct interdependence. It is the present purpose to determine

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whether or not a correlation exists among the following factors: number of injections, intervals and duration, titer of the circulating antibody, and histologic alteration in the thyroid.

METHODS

The methods have been described in detail in earlier publications [1-3] and will only be summarized here.

The thyroid glands were excised, quickly frozen in dry ice and stored in this state. In some experiments, hemithyroidectomy was performed in order to investigate the effect of autologous inoculation upon the remaining gland tissue. In other instances, glands from many animals, sacrificed by exsanguination, were pooled. Before use, gland substance was thawed, trimmed of extraneous tissues, minced, and homogenized with an equal amount (w/v) of ice cold saline (buffered with phosphate salts to pH 7.2), using a Teflon homogenizer of the Potter type. After brief centrifugation, the crude extract was combined with an equal volume of Freund adjuvant and introduced intradermally into the footpads of the animals. Each inoculum contained 0.05 ml. of the emulsion.

As noted below, many different immunization schedules were used. On occasion, a purified thyroglobulin, prepared by Dr. S. Shulman, was injected instead of the crude extract.

The circulating thyroid antibodies in the serum of experimental animals were measured by means of precipitation, complement fixation and tanned cell hemagglutination tests. The latter procedure is a modification of the test originally devised by Boyden.[8] It consisted of treating human erythrocytes with highly diluted tannic acid and coating the tanned cells with thyroid extract. The coated tanned red blood cells were added to serial dilutions of the antiserum. The titer of the antiserum was measured as the greatest serum dilution showing definite agglutination.

Samples of all or part of the thyroid gland and of control tissue from various organs were immediately fixed in buffered formaldehyde, embedded in paraffin and stained with hematoxylin and eosin. Other staining methods used on occasion were Masson's trichrome, periodic acid-Schiff, Perls's Prussian blue and the Ciaccio stain [9] for lipids. All sections were examined without knowledge of immunologic data. The thyroid tissue available for histologic study was, in general, adequate for numerous sections. In a few instances, following hemithyroidectomy, part of the remaining lobe was also used for determining its antigen content, leaving less material for histologic study. In all cases, however, cross sections in the vertical or transverse axis of the thyroid were available.

The thyroid alterations were graded in accordance with the extent

of the interstitial inflammatory reaction and the reduction in size and colloid content of the follicles. The lesion designated as one plus (+) consisted of a few small interstitial infiltrates of lymphoid cells, and, occasionally, of a few fibroblasts with slight fibrosis unrelated to the lymphocytic infiltrates; these were usually seen about small thyroid follicles. The bulk of the thyroid tissue was normal, and there was no conspicuous desquamation of cells into the lumens of the follicles. Lesions designated as two plus (++) were moderate to severe in intensity and were characterized by prominent inflammatory infiltrations, involving, in scattered or in diffuse fashion, the interstices and numerous follicles or the entire lobe.* The exudate contained lymphocytes, plasma cells, eosinophils and neutrophils in varying proportions. Within follicles were degenerated epithelial cells, macrophages with or without hemosiderin, and, frequently, disintegrating leukocytes. The colloid was altered in varied fashion; it was liquefied, transformed into small globules or appeared as granular debris. Faint outlines of degenerated cells were common in such follicles. In some instances in the dog and rabbit, moderate fibrosis was observed.

The sections from control animals and from the thyroid glands designated as normal, exhibited the well known placid colloid follicular pattern as a uniform feature. There were neither interstitial inflammatory infiltration nor conspicuous alteration in the appearance of the colloid. Rare desquamated epithelial cells within normal-appearing colloid follicles were not considered to be of pathologic significance.

Experimental Observations

The results obtained in the entire series of experiments are shown in Tables I to XI. The controls, 30 rabbits and 5 guinea pigs, are listed in Table I. In all of these, the blood was tested for homologous antithyroid antibody. Table I also indicates the results obtained in 6 rabbits and 7 guinea pigs which had been given injections of adrenal extract, and 3 rabbits which had received injections of pituitary extract. Unless otherwise stated, all inoculums used in the controls were introduced intradermally in combination with the complete Freund adjuvants. Tables II to VI and XI represent the data obtained from immunization with autologous or homologous thyroid extract in 15 dogs, 67 rabbits and 27 guinea pigs. Tables VII to X record the results of experiments with heterologous thyroid extract in 41 rabbits.

The control animals clearly revealed the absence of thyroid-specific antibody and the absence of histologic abnormalities in the thyroids.

* This method of grading is not the same used in reference 5.

TABLE I

CONTROLS

No. of animals used	Inoculum	No. of injections	Method of injection	Duration (mo.)	Antibody titer *	Histologic alteration
Rabbits						
5	Pooled homologous serum	1		3	0	0
1	Normal human serum	1		1	0	0
1	Normal hog serum	1		1	0	0
3	Pooled rabbit testicular extract	2		1	0	0
8	Pooled rabbit pancreatic extract	2		5 to 7	0	0
5	Complete adjuvants with buffered saline	1		3	0	0
3	Complete adjuvants without buffered saline	3		3	0	0
2	Homologous thyroid extract without adjuvants	2	i.d.	2	0	0
		1	i.v.			
1	Homologous thyroid extract without adjuvants	2	i.d.	10	0	0
		9	i.v.			
1	Human pancreatic cancer extract	multiple	i.v.	7	0	0
Guinea pigs						
5	Homologous pancreatic extract			4	0	0
		Additional controls				
Rabbits						
2	Autologous adrenal extract			1 and 2		0
2	Homologous adrenal extract			1 and 2		0
1	Autologous and homologous adrenal extract			1		0
1	Human adrenal extract			1		0
3	Homologous pituitary extract			2 to 2.5		0
Guinea pigs						
6	Homologous adrenal extract			2 to 4		0
1	Beef adrenal extract			1		0

i.d.—intradermal.
i.v.—intravenous.

* Tested against homologous (rabbit or guinea pig) thyroid extract.

Pooled rabbit, human, and hog serums, extracts of homologous testis, pancreas, adrenal, pituitary, and of human pancreatic cancer failed to alter the thyroid structure in any way. The complete Freund adjuvants used without the addition of any extract gave negative results. This was also the case with pooled homologous thyroid extract, when injected

TABLE II

EFFECT OF AUTOLOGOUS AND HOMOLOGOUS THYROID EXTRACT IN THE DOG

Dog no.	Inoculum	No. of injections	Duration (mo.)	Antibody titer	Histologic alteration
1184	PTE	3	8	0	++
1186	ATE	1	1*	0	0
1196	ATE	1	8	0	++
	PTE	3			
1198	ATE	1	8	0	0
	PTE	3			
1514	PTE	8	29	20	++
1600	PTE	8	29	80	++
1609	PTE	8	29	0	+
1614	PTE	8	29	80	++
1620	PTE	1	1*	0	0
1622	PTE	1	1*	0	0
1519	PTE	1	1*	not examined	+
1191	†	0	8	0	0
1623	PTE	1	3	0	(right lobe) +
	PTE	8	26	320	(left lobe) ++
1521	PTE	4	11	0	(left lobe) +
	PTE	6	22	320	(right lobe) +
1618	PTE	5	16	320	(left lobe) ++
	PTE	8	24	320	(right lobe) ++

PTE = pooled thyroid extract.
ATE = autologous thyroid extract
* Died
† Hemithyroidectomy, no injections given.

without the addition of the Freund adjuvants. Moreover, in 12 guinea pigs, homologous extract of pancreas or adrenal and heterologous adrenal extract had no effect on the thyroid.

Dog Investigations

Table II represents the effects of active immunization of 15 dogs with homologous thyroid extract. In 10 there was slight or marked thyroiditis. Severe lesions were produced in the glands of two dogs (nos. 1184 and 1196) by pooled dog thyroid extract alone and by a combination of autologous and homologous extract (Figs. 1 and 2). Figures 3 and 4 clearly show the difference in the histologic alteration in the right and

left lobes of the same dog (no. 1623), after 3 and 26 months, following 1 and 8 injections of homologous extract, respectively.

Four dogs died 4 weeks after a single injection of pooled homologous thyroid extract. No antibody was detected in the blood of 3 of these, and their thyroid glands were normal. In the thyroid of the fourth dog, slight histologic alterations were present. These were comparable to those shown in Figure 3. The blood of this dog was not tested for antibodies.

The conclusions drawn from these experiments are as follows:

Slight alterations were observed in the thyroids of dogs immunized with pooled homologous extract over a period of 1 to 11 months. These changes, however, were not accompanied by the appearance of detectable antibody in the blood. Repeated supplementary inoculations (5 to 8) caused antibodies to appear 16 to 26 months after the first injection. In a single dog there was a change from slight thyroiditis 3 months after a single injection, to a severe thyroiditis after 7 supplementary injections in the course of 23 months. The change in the lesion was paralleled by a rise of antibody titer from 0 to 320. Another dog, in which the left and right lobes were examined after an interval of 11 and 22 months, respectively, exhibited no significant change in the degree of thyroiditis. This, despite the fact that antibody was absent after 11 months and appeared in a titer of 320 at 22 months; there had been 2 supplementary inoculations in the interval. In a third dog, both titer levels were the same, and the lesions in left and right lobes were very similar after 5 and 8 injections and a lapse of 16 and 26 months, respectively. On the other hand, unusually severe thyroid lesions after repeated injections with pooled thyroid extract in 3 dogs were at no time accompanied by a demonstrable antibody titer when tested after 8 and 29 months. Hemithyroidectomy alone, not followed by injection of extract, resulted in neither the production of thyroid-specific antibodies nor of histologic lesions in the remaining lobe after a period of 8 months.

Cross circulation experiments in 5 additional dogs (not listed in Table II) which received donor blood from 5 dogs with antithyroid antibody titers of 80 to 160, also gave negative results. In the recipient dogs, the transferred antibody disappeared rapidly, and the thyroids, when examined 2 to 6 months later, revealed no alterations.

Rabbit Investigations

Table III represents the results of a single injection of autologous and homologous rabbit thyroid extract. In the absence of antibody, there were no alterations of thyroid structure in two animals. Distinct histologic lesions were observed at relatively low titers (640) as well as at 10 times higher levels, regardless of whether the animals were sacrificed

at 2 or at 5 months. In the presence of antibody, moderate to severe thyroiditis occurred within 2 months of a single injection of autologous or homologous thyroid extract (Figs. 7 and 8). The minor lesions in the

TABLE III

EFFECT OF AUTOLOGOUS AND HOMOLOGOUS THYROID EXTRACT IN THE RABBIT

Rabbit no.	Inoculum	No. of injections	Duration (mo.)	Antibody titer	Histologic alteration
202	ATE	1	3	1250	0
203	ATE	1	3	640	++
205	ATE	1	3	1250	+
206	ATE	1	3	640	++
207	ATE	1	3	0	0
209	ATE	1	3	0	0
210	ATE	1	5	1250	+
212	ATE	1	5	640	0
214	PTE	1	4.5	640	++
215	PTE	1	2.5	6250	++
216	PTE	1	2	640	++
217	PTE	1	5	6250	++
218	PTE	1	4.5	6250	++
219	PTE	1	2.5	6250	++
220	PTE	1	2.5	6250	++
221	PTE	1	2.5	6250	++
223	PTE	1	2.5	640	++
224	PTE	1	2.5	1250	0

ATE = autologous thyroid extract
PTE = pooled thyroid extract

thyroid of 2 rabbits with intermediate titer ranges were of the same intensity after 3 and 5 months. In 3 animals, however, despite the presence of circulating antibody, the microscopic pattern was that of normal thyroid.

In Table IV the effects of repeated injections (2 to 4) with homologous thyroid extract are listed. In this series of experiments, severe histologic lesions were more regularly observed in association with higher antibody titers (320 to 3125). Figures 5, 6, 9 and 10 are representative examples of thyroid alterations encountered in this group. Minor lesions were accompanied by lower levels of antibody (16 to 160). In 3 animals without circulating antibody, the thyroid glands were essentially normal; one rabbit had evidence of mild thyroiditis only. On the other hand, the thyroid was normal in 4 rabbits with both low and high antibody titers. The time factor and the number of injections seemed to have no influence on titer level or on the extent of histologic alteration. Slight and marked lesions were of the same intensity after 2, 3 or 4 injections and after intervals of 3½, 7 and 13 months.

When purified rabbit thyroglobulin was used as the immunizing agent,

TABLE IV

EFFECT OF REPEATED INJECTIONS WITH HOMOLOGOUS THYROID EXTRACT IN THE RABBIT

Rabbit no.	Inoculum	No. of injections *	Duration (mo.)	Antibody titer	Histologic alteration
288	PTE	2	3.5	80	+
289	PTE	2	3.5	640	++
290	PTE	2	3.5	625	++
292	PTE	2	3.5	125	o
293	PTE	2	3.5	o	o
294	PTE	2	3.5	3125	++
297	PTE	2	2.5	40	o
298	PTE	2	3.5	3125	o
299	PTE	2	3.5	3125	++
401	PTE	2	3.5	3125	o
402	PTE	2	3.5	1250	++
555	PTE	4	13	80	+
556	PTE	4	13	16	+†
557	PTE	4	13	o	o
558	PTE	3	7	1280	++
559	PTE	3	13	160	+
560	PTE	3	13	320	++†
561	PTE	3	13	o	o
562	PTE	2	5	o	+
563	PTE	2	13	40	+

PTE = pooled thyroid extract.

* All injections given at 2-month intervals.

† Cytoplasmic swelling.

TABLE V

EFFECT OF PURIFIED HOMOLOGOUS RABBIT THYROGLOBULIN

Rabbit no.	Inoculum	No. of injections	Duration (mo.)	Antibody titer	Histologic alteration
417	PRTG	1	3	2560	++
418	PRTG	1	3	729	++
419	PRTG	1	2	2560	++
420	PRTG	1	2.5	243	++
421	PRTG	1	2.5	2187	+
422	PRTG	1	2.5	6561	++

PRTG = purified rabbit thyroglobulin.

(Table V) the result was uniformly positive in all 6 rabbits tested. The titer of antibody, however, was not the determining factor in respect to the severity of thyroiditis. The histologic alterations were as severe at

TABLE VI

EFFECT OF A SINGLE INJECTION WITH AUTOLOGOUS EXTRACT FOLLOWED BY ONE OR TWO INJECTIONS WITH HOMOLOGOUS EXTRACT

Rabbit no.	No. of PTE injections *	Duration (mo.)	Antibody titer	Histologic alteration
464	2	9	160	++
465	1	6	40	++
467	2	9	45	o
468	2	9	135	++
469	1	6	405	++
471	1	6	1215	++
472	1	6	405	++
473	2	9	405	+
485	1	3.5	125	+
486	1	3.5	625	+
487	1	3.5	625	+
488	1	3.5	125	++
489	1	3.5	625	+
475	1†	3.5	o	o
476	1	3.5	o	o
477	1	3.5	25	+
478	1	3.5	25	o
479	1	3.5	o	o
480	1	3.5	25	+
481	1	3.5	o	+
482	1	3.5	125	++
483	1	3.5	25	+
484	1	3.5	o	o

* Injections of PTE (pooled thyroid extract) were given after a single injection of autologous thyroid extract.

† Tubercle bacilli omitted from Freund adjuvants in injections to rabbits 475 to 484.

low titers of antibody (243) as at titers of 20 times as high, and were less marked at an intermediate titer range.

Table VI exhibits the combined effect of autologous and homologous thyroid extract. In the experiments listed in the lower half of the table, tubercle bacilli were omitted from the Freund adjuvants. In the animals sacrificed after 3½, 6 and 9 months, the intensity of thyroiditis did not differ significantly in relation to the time factor. Nor was there a correla-

tion of the titer levels with respect to the duration of the experiments or the degree of thyroiditis. In fact, the only animal in this series with a histologically normal thyroid had an antibody titer as low (45) as that in one with severe thyroiditis.

When the tubercle bacilli were omitted from the adjuvant, the histologic alterations were only slight and the titers were low (25), except in one animal in which a higher titer (125) was accompanied by distinct histologic lesions. In 4 of 10 animals, there were neither circulating antibodies nor evidences of histologic abnormality. While in this group the antibody levels were considerably below those observed in the rabbits listed in Tables III and V, the omission of the tubercle bacilli from the adjuvant might have caused the depressant effect on antibody formation. The omission resulted in less marked or absent histologic alterations in the thyroid.

The data recorded in Tables III to VI may be summarized briefly as follows:

Fifty-seven rabbits received injections of rabbit thyroid extract or purified rabbit thyroglobulin, in each instance combined with the complete Freund adjuvants. Thyroiditis occurred in 45, more than three fourths of the total. The histologic alterations correlated with positive antibody titers in 41 and the existence of normal thyroid with negative titers in 5; a parallelism thus was found in 80.7 per cent of the animals. In 8 animals with elevated titers, there was no histologic alteration. The correlation of thyroiditis with the presence of antibody in the blood, however, was not quantitative. Slight or severe histologic alterations did not regularly correlate with low or high antibody titers. Nor did the degree of thyroiditis have any relation to the duration of the experiment (time factor). It was concluded that the thyroiditis brought about by immunization with autologous and homologous thyroid extract was not necessarily progressive, even in the presence of a high circulating antibody titer.

The results of immunization of rabbits with heterologous extract, prepared from hog, dog, beef and human thyroid tissue, are listed in Tables VII to X.

Eight rabbits received a single intradermal injection with hog thyroid extract and were sacrificed after 1½ to 8½ months. In only one of these, sacrificed after 8 months, was there a slight thyroiditis; this was accompanied by a relatively low antibody titer (80). In the remaining 7 rabbits, the thyroid was normal histologically although the antibody titer ranged between 40 and 135 in animals sacrificed after 2 months and between 20 and 80 in animals sacrificed after 3½ to 8½ months.

Following repeated intravenous injections of purified hog thyro-

TABLE VII

EFFECT OF HETEROLOGOUS HOG THYROID EXTRACT, PURIFIED HOG THYROGLOBULIN AND THYRO-ALBUMIN

Rabbit no.	Inoculum	No. of injections	Method of injection	Duration (mo.)	Antibody titer *	Histologic alteration
423	HTE	1		2	45	o
424	HTE	1		2	135	o
522	HTE	1		8.5	80	+
523	HTE	1		8.5	20	o
524	HTE	1		3.5	40	o
525	HTE	1		3.5	20	o
526	HTE	1		1.5	40	o
527	HTE	1		1.5	40	o
674	PHTG	1		1	243	o†
677	PHTG	1		1	243	+
613	PHTG	multiple	i.v.	2.5	19683	+†
614	PHTG	multiple	i.v.	3	19683	+
676	PHTG	1 multiple	i.d. i.v.	5	729	+†
678	PHTG	1 multiple	i.d. i.v.	5	2187	++
642	PHTA	1 multiple	i.d. i.v.	3	o	o
643	PHTA	1 multiple	i.d. i.v.	3	o	o

HTE = hog thyroid extract.
PHTG = purified hog thyroglobulin.
PHTA = purified hog thyralbumin.
i.v. = intravenous.
i.d. = intradermal.

* Tested against rabbit thyroid.
† Cytoplasmic swelling.

TABLE VIII

EFFECT OF HETEROLOGOUS DOG THYROID EXTRACT IN THE RABBIT

Rabbit no.	Inoculum	No. of injections	Method of injection	Duration (mo.)	Antibody titer *	Histologic alteration
546	CTE	1	i.d.	2	o	o
663	CTE	1	i.d.	1	729	o
664	CTE	1	i.d.	3	243	o
665	CTE	1	i.d.	1	81	+†
666	CTE	1	i.m.	1	o	o†
667	CTE	1	i.m.	3	o	o
668	CTE	1	i.m.	1	o	+†

CTE = canine thyroid extract.
i.d. = intradermal.
i.m. = intramuscular.

* Tested against rabbit thyroid.
† Cytoplasmic swelling.

TABLE IX

EFFECT OF HETEROLOGOUS BEEF THYROID EXTRACT IN THE RABBIT

Rabbit no.	Inoculum	No. of injections	Duration (mo.)	Antibody titer *	Histologic alteration
507	BTE	1	6	320	o
508	BTE	1	6	80	+
509	BTE	1	6	320	o
510	BTE	1	6	160	o
511	BTE	1	6	160	o
512	BTE	1	6	1280	+

BTE = beef thyroid extract.
* Tested against rabbit thyroid.

TABLE X

EFFECT IN THE RABBIT OF HETEROLOGOUS EXTRACT OF NORMAL, COLLOID NODULAR, AND CANCEROUS HUMAN THYROID

Rabbit no.	Inoculum	No. of injections	Method of injection	Duration (mo.)	Antibody titer *	Histologic alteration
530	HTE	1		2	o	o
531	HTE	1		3.5	o	o†
532	HTE	1		3	o	o
533	HTE	1		3.5	o	o
536	HTE	1		3.5	27	o
537	HTE	1		3.5	27	o
540	HCGE	1		3	81	o
541	HCGE	1		3	81	o
538	HTCaE	1	i.d.	3	o	o
		1	i.p.			
539	HTCaE	1	i.d.	3	o	o†
		1	i.p.			
		multiple	i.v.			
542	HTCaE	1	i.d.	4	o	o
		1	i.p.			
		multiple	i.v.			
543	HTCaE	1	i.d.	4	o	o†
		1	i.p.			
		multiple	i.v.			

HTE = human thyroid extract.
HCGE = human colloid goiter extract.
HTCaE = human thyroid cancer extract.
i.d. = intradermal.
i.p. = intraperitoneal.
i.v. = intravenous.
* Tested against rabbit thyroid
† Cytoplasmic swelling.

globulin, a very high anti-rabbit thyroid antibody titer was found in the blood of 2 rabbits when sacrificed after 3 months. Despite this, the histologic alterations were of relatively slight degree. Only one of the animals (no. 678), with an intermediate titer range, had severe thyroiditis. Repeated intravenous injections of hog thyro-albumin gave entirely negative results.

Extracts of dog thyroid were injected intramuscularly into 3 rabbits. In only one of these, sacrificed one month later, was there slight thyroiditis. No anti-rabbit thyroid antibodies appeared in the blood of any of these 3 animals. A single intradermal injection of the same extract gave somewhat similar results. Very slight thyroiditis accompanied relatively low antibody titers. In 2 animals with higher titer levels, there was no evidence of thyroiditis at all.

When beef extract was injected, slight thyroiditis appeared in 2 rabbits. This was accompanied by a very low titer in one animal and by a very high one in the other. The thyroids were normal in the 4 remaining animals with intermediate titer ranges (2 to 4 times the lowest titer).

While the administration of extracts from normal human thyroid and from benign colloid goiter resulted in very low antibody titers against rabbit thyroid, no antibody was detected in 4 rabbits receiving saline extract of cancerous thyroid. In none of the rabbits of this series were there evidences of thyroiditis.

The data listed in Tables VII to X may be summarized as follows:

The injections into rabbits of heterologous extracts prepared from the thyroids of beef, hog, and dog were followed in one or two instances in each group by slight thyroiditis. The lesions were paralleled by low antibody titers (80) when tested against rabbit thyroid, except in one animal with a high titer range (1280). None of the other rabbits immunized with heterologous thyroid extracts, including that procured from human thyroid, exhibited evidence of histologic alteration.

Distinct thyroiditis was present, however, in 5 rabbits, following repeated intravenous injections with purified hog thyroglobulin. These lesions developed in the course of 1 to 5 months. Figure 11 represents the lesion in one of the rabbits; the histologic changes in the remainder were less marked.

In addition to the histologic patterns of thyroiditis thus far described, there was also a peculiar, foamlike swelling of the follicular epithelial cytoplasm. This was observed in some of the rabbit glands after the combined injection of autologous and pooled homologous extracts. The inflammatory lesions in these instances were comparatively slight and appeared unrelated to the epithelial swelling. The foamy alteration was even more marked following injections of dog thyroid extract, after re-

peated intravenous injections of purified hog thyroglobulin and extracts of both normal and cancerous human thyroid tissue (Fig. 13). Since only paraffin sections were available, conventional fat stains on frozen sections were not possible. The Ciaccio stain, however, failed to demonstrate the presence of lipid. The peculiar swelling resembled a process of hydration or storage and was combined in one instance with distinct cytolysis of follicular epithelium (Fig. 14).

Guinea Pig Investigations

Table XI combines the results in 2 groups handled in slightly different manner. In the first, 19 guinea pigs received intradermal injections of pooled homologous thyroid extract. The histologic alterations in the thyroids of these animals were for the most part comparatively slight. In 10 of the 19, there was no detectable antibody against guinea pig thyroid.

The 8 guinea pigs listed in the lower part of the table first received injections into the skin of the neck region, and, 2 months later, into the footpads; in each instance the inoculum was pooled guinea pig thyroid extract.

While these animals uniformly showed significant thyroiditis, there was no quantitative correlation between this and the amount of circulating antibody. An unusually high titer (3125) was accompanied by comparatively minor lesions, and in guinea pigs with severe thyroiditis, the antibody titers were as low as 25 and did not exceed 125.

The lesions in the guinea pigs were similar to those observed in the rabbits. Hemosiderin granules were occasionally seen in the colloid, and clumps of erythrocytes appeared in a few follicles; in a few instances, these were engulfed by macrophages. The interstitial inflammatory reaction and the reduction in size of colloid-bearing follicles were as marked as in the rabbit lesions (Fig. 12).

One of the guinea pigs (Table XI) was pregnant. In this animal, there was only slight focal lymphocytic infiltration in the thyroid and no detectable antibody in the blood. The thyroids of its 3 offspring were normal.

Discussion

In the dog, rabbit, and guinea pig, each immunized with species-homologous thyroid extract, the histologic lesion of thyroiditis was of a similar pattern. It was characterized by a reduction in the size of colloid-filled follicles and an infiltration by inflammatory cells of varied types. Differences existed not only in the extent of these lesions, especially in the rabbit and guinea pig, but also in the cellular composition of the exudates. The accumulation of lymphocytes and plasma cells and—

especially in the rabbit—eosinophils, was a striking feature. Focal infiltrations were present in all affected glands, even if the bulk of the thyroid tissue was not significantly altered. The lumens of the follicles,

TABLE XI

EFFECT OF HOMOLOGOUS THYROID EXTRACT IN THE GUINEA PIG

Guinea pig no.	Inoculum	No. of injections	Duration (mo.)	Antibody titer	Histologic alteration
1	PGTE	2*	7.5	20	+
2	PGTE	2	7.5	40	++
3	PGTE	2	7.5	80	+
4	PGTE	2	7.5	40	+
5	PGTE	2	7.5	160	+
6	PGTE	1	7.5	0	+
7	PGTE	1	7.5	80	+
8	PGTE	1	7.5	0	+
9	PGTE	1	7.5	160†	+
10	PGTE	1	7.5	0	+
11	PGTE	1	7.5	0	+
12	PGTE	1	7.5	80	+
13	PGTE	2	7.5	0	+
14	PGTE	2	7.5	0	+
16	PGTE	2	7.5	0	+
17	PGTE	2	7.5	81	+
18	PGTE	1	7.5	0	+
19	PGTE	1	7.5	0	+
20	PGTE	1	9.5‡	0	+
78	PGTE	2	3.5	3125	+
80	PGTE	2	3.5	25	++
81	PGTE	1	2.5	25	+
83	PGTE	1	2.5	25	++
86	PGTE	2	5.5	125	++
87	PGTE	2	5.5	125	+
88	PGTE	2	2.5	125	++
90	PGTE	2	2.5	25	++

PGTE = pooled guinea pig thyroid extract.
* Where a second injection was given, it was after a 2 month interval.
† Positive skin test.
‡ Pregnant; 3 offspring revealed no histologic alteration.

adjacent to denser infiltrations, contained various cells: macrophages (viable or in the process of disintegration), eosinophils (especially in the rabbit), neutrophils (particularly in the dog and rabbit), and

lymphocytes in all 3 species. The homogeneity of the colloid disappeared and was replaced in part by globular particles or granular debris. The follicular epithelium showed vacuolar degeneration and desquamation. Disintegration of various cells within the follicles was a prominent feature. Disruption of the follicle wall was occasionally seen, especially in the rabbit and guinea pig, and was accompanied by cytolytic alterations in the epithelium. Regenerative activity was suggested by a few solid tubules of thyroid epithelium containing mitotic figures. Focal fibrosis was also encountered in a few instances, especially in the dog and rabbit. The occasional presence of hemosiderin-containing cells in the densely inflamed stroma or within intact thyroid follicles clearly pointed to extravasation of erythrocytes. The site of inoculation was such that there was no trauma to the thyroid in these animals.

The thyroiditis was brought about experimentally in these animals by one or repeated intradermal injections of homologous thyroid extract. In the initiation of the thyroiditis, an interaction between a thyroid-specific antibody and its selective target, the colloid-bearing follicles, was the foremost pathogenetic factor. No lesions were encountered in any other organ examined in these animals, except for a slight or moderate degree of interstitial pyelonephritis in 11 guinea pigs and in 2 rabbits. These lesions, we feel, were not related to the immunization procedure. Tissues examined were parathyroid, adrenal, heart, spleen, lung, liver, kidney, pancreas, testis, ovary and, in several dogs and rabbits, the pituitary.

The failure of several rabbits to exhibit evidence of thyroiditis, despite the presence of specific thyroid antibodies in the blood (Tables III and IV), has not been satisfactorily explained. Perhaps the paucity of thyroid tissue available for histologic examination in this group might have introduced an artificial factor of technical nature. In some of the animals, only a few sections were available. Small focal inflammatory infiltrations might have been present in portions of the thyroid not examined; considerable tissue was used in testing for thyroid antigen content. In two rabbits, in which the initial preparations were negative, additional sections were procured, and these revealed focal inflammatory lesions of moderate severity.

In two dogs (Table II), with marked thyroiditis, no circulating antibody was detected. It might be assumed that the antibody had disappeared when these dogs were sacrificed. Whether or not the destruction of normal thyroid parenchyma was responsible for its disappearance cannot be determined. The two animals were not tested until shortly before they were sacrificed.

There were 10 guinea pigs (upper part of Table XI) with slight

thyroiditis and without detectable antibody. In general, the antibody titers against guinea pig thyroid were low in this series, ranging between 20 and 80.

That at least part of the thyroid-specific antibody can combine with the intrafollicular colloid is suggested by the positive labeling with fluorescent antibody in frozen sections of normal rabbit thyroid previously acted upon by rabbit auto-antiserum to thyroid. These data, which have been published recently,[10] include the serums from two rabbits listed in Table IV. The histologic lesion in one of these rabbits is illustrated in Figure 10. The presence of macrophages and lymphocytes in a distended lymphatic is of particular interest here since it might represent the response to colloid resorption. Recently, under similar experimental conditions, we have observed well stained colloid surrounded by numerous lymphocytes within a lymph vessel of the thyroid. This rabbit had received 3 injections of pooled rabbit thyroid extract and was sacrificed after 3 months. There was moderately severe focal thyroiditis in the periphery of the gland close to the colloid-containing lymphatic; in this instance the titer of auto-antibody was 7,290.

Various attempts to produce thyroiditis in experimental animals by vitamin deficiencies and by the administration of thiouracil and radioactive iodine have been summarized by Hellwig and Wilkinson.[11] Using irradiated iodine, these authors succeeded in confirming the experiments of Goldberg, Chaikoff, Lindsay and Feller,[12] Gorbman,[13] and of Maloof, Dobyns and Vickery,[14] who produced thyroiditis in white rats and guinea pigs by injecting 1 mc. of I^{131} intraperitoneally. In the course of 4 weeks, the thyroid glands in these animals were replaced by fibrous tissue. The intermediate phases observed by Hellwig and Wilkinson after 2, 4 and 7 days bore some resemblance to the lesions observed in our animals, although the latter had developed in the course of several months following injection of thyroid extract. Hellwig and Wilkinson emphasized that disruption of the follicle wall due to epithelial degeneration resulted in the exposure of the mesenchymal stroma to colloid. Kracht[15] induced a diffuse lymphocytic thyroiditis within 7 days by injecting an ether soluble extract of human thyroid colloid directly into the thyroid of guinea pigs. The lipid protein complexes contained within the extract were considered the agents responsible for the extensive inflammation and foreign body granulomas.

Whatever factors might affect the normal equilibrium between secretion and resorption of colloid, any damage to the lining epithelium or to the follicle content could disturb this balance. Alterations in the colloid itself and in the cytoplasm of follicle epithelium, effected in our experiments by intradermal injection of autologous and homologous thyroid

extract, appeared to be an organ-specific immune response. In a few instances, even heterologous thyroid extract has acted in a similar manner. Various factors, such as the intensity of inflammatory reaction, hemorrhage, the action of macrophages in the resorption of blood and altered colloid, and the regenerative capacity of affected thyroid follicles, apparently contribute to the eventual outcome of the immune response. Variations in the degree of follicle damage and the resulting inflammation are probably responsible—under controlled experimental conditions —for the persistence or disappearance of the circulating thyroid-specific antibody.

From the manner in which these experiments have been carried out, it would be somewhat speculative to postulate a regular sequence in the various histologic alterations. Individual differences in reactability of the animals, moreover, did not permit strict correlation of the structural alterations with the duration of the experiment. Nor was the severity of the lesions necessarily dependent upon the level of the specific antithyroid antibody in the blood of the immunized animals.

Summary

Thyroiditis has been induced in rabbits, guinea pigs, and dogs by one or more intradermal injections of homologous saline thyroid extract, purified thyroglobulin and, in a few instances, in rabbits only, by heterologous thyroid extract. In all 3 species, the histologic lesions were of a similar type, characterized by a reduction in size and colloid content of the thyroid follicles and by inflammatory reactions indicative of thyroiditis.

The correlation between the intensity of thyroiditis and the level of circulating thyroid-specific antibody titer in the immunized animals was, in general, qualitative rather than quantitative. Correlation was greatest in the rabbit (about 80 per cent), especially when homologous extract alone, or combined with autologous extract, was used.

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[*Illustrations follow*]

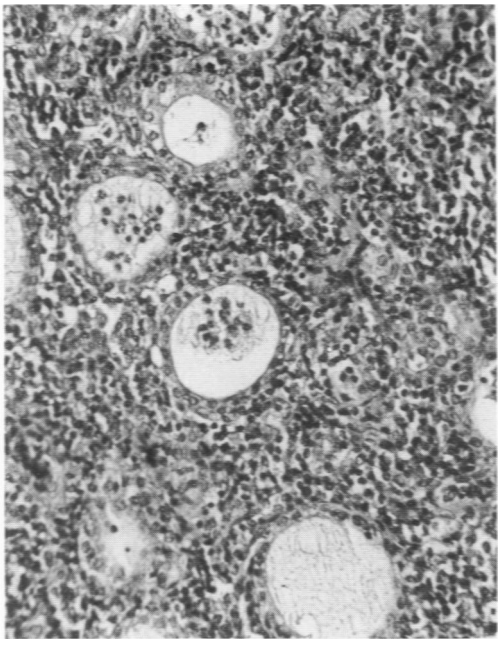
LEGENDS FOR FIGURES

Except where indicated, sections illustrated were stained with hematoxylin and eosin.

FIG. 1. The remaining lobe of a dog (no. 1169) which had first received extract prepared from its own thyroid (hemithyroidectomy) and, later, pooled dog thyroid extract. The animal was sacrificed after 8 months. The normal follicular pattern is obscured by a dense inflammatory reaction. The preserved thyroid follicles contain liquefied colloid infiltrated by leukocytes and macrophages. Other follicles are completely masked by infiltration of neutrophils, lymphocytes, plasma cells, and macrophages. × 200.

FIG. 2. Dog (no. 1184) injected 3 times with pooled dog thyroid extract and sacrificed after 8 months. Small and nearly solid follicles, with little colloid, constitute a striking feature. The diffuse inflammatory exudate contains many leukocytes. × 96.

FIGS. 3 and 4. Right and left lobes of dog (no. 1623) listed in Table II. The right lobe was removed after 3 months, following a single injection of pooled dog thyroid extract; the left lobe, after 26 months. Figure 3 shows a normal colloid pattern with slight interstitial inflammation in the center and desquamated cells within adjacent colloid follicles. This was the only lesion in the lobe. Distention and hyperemia of two veins, at the lower left and in the right upper corner, are caused by ligation, incident to the removal of the lobe. In Figure 4, the inflammatory exudate is diffuse. Dense cellular exudate appears in the interfollicular stroma and within numerous follicles. There is marked disintegration of leukocytic nuclei within the follicles. The entire lobe was involved uniformly. × 200.



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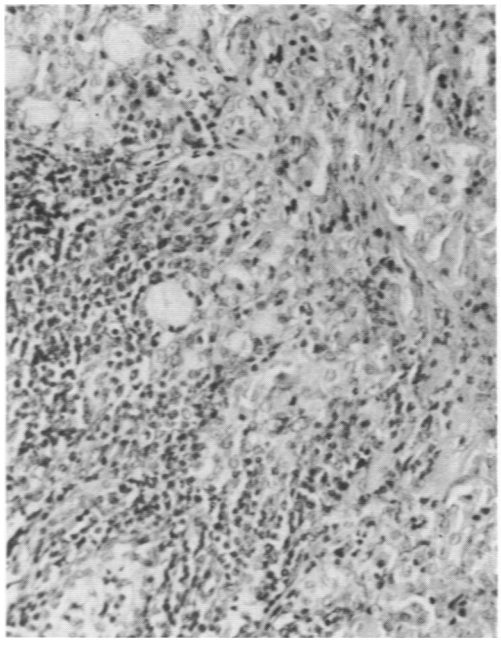

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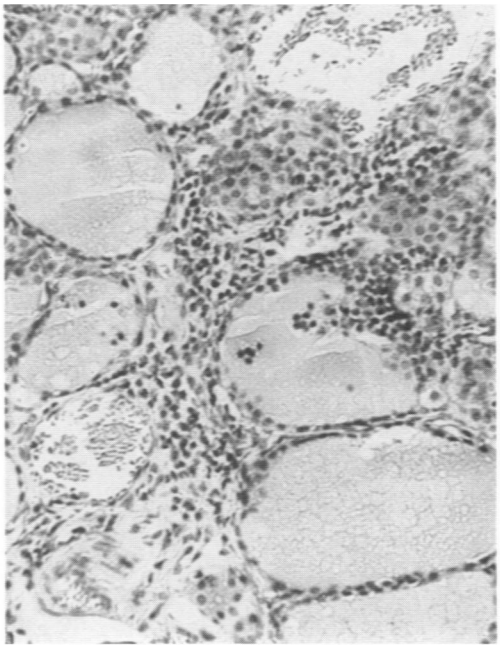

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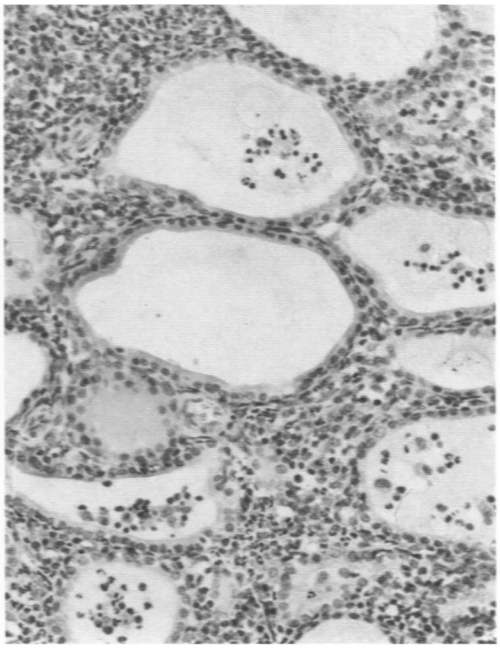

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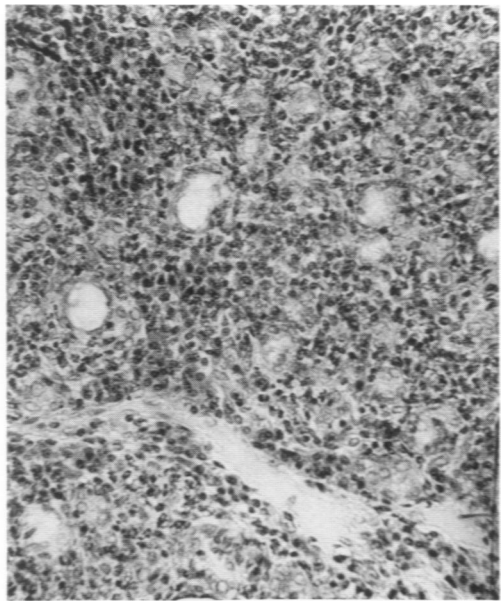
FIG. 5. Rabbit (no. 402) received 2 intradermal injections of pooled rabbit thyroid extract, 2 months apart, and was sacrificed after 3½ months. Antibody titer against rabbit thyroid, 1:1250. Numerous plasma cells and eosinophils infiltrate the interstitial tissue. The thyroid follicles are of small size. There are many disintegrating leukocytes in the more or less masked follicle lumens. × 200.

FIG. 6. Rabbit (no. 200). The experimental procedure as in rabbit no. 402 (Fig. 5). Titer, 1:625. Interstitial tissue is infiltrated by leukocytes and a few plasma cells in the lower field to the right. The single macrophage in the follicle to the left contains hemosiderin granules. Note also the vacuolated appearance of the colloid and scattered macrophages and leukocytes in several follicles. × 200.

FIGS. 7 and 8. Rabbit (no. 214) received a single intradermal injection of pooled rabbit thyroid extract and was sacrificed after 4½ months. Titer, 1:640. Figure 7 clearly shows the disruption of the follicle wall, detached epithelium and dense infiltrations with lymphocytes and macrophages. In Figure 8 the interstitial infiltration consists of scattered lymphocytes and neutrophils. There are numerous disintegrating cells in the follicles; these represent ghost-like remnants of desquamated cells in the faintly staining colloid. × 200.



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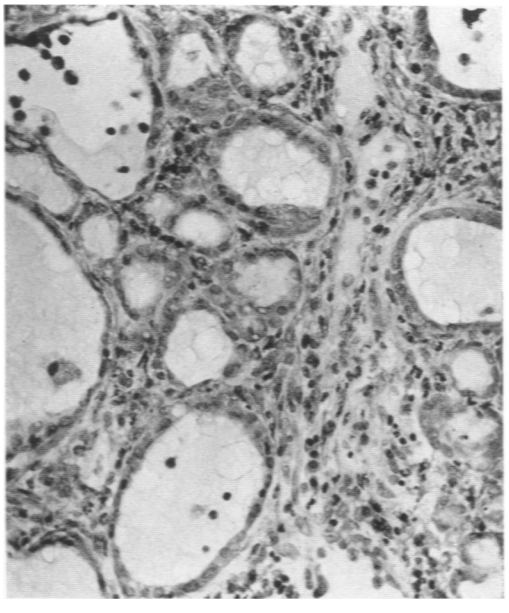

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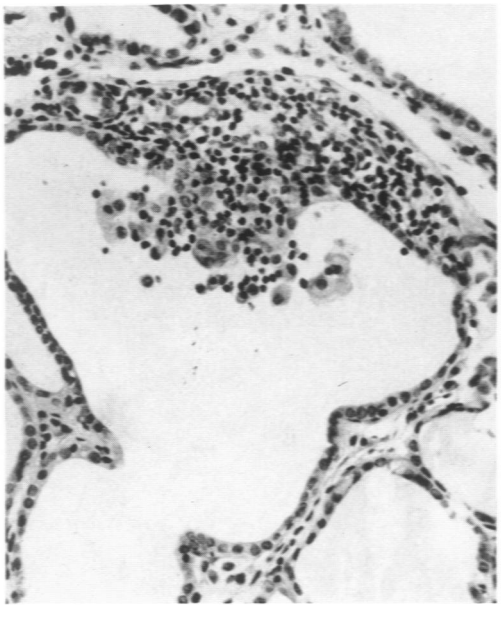

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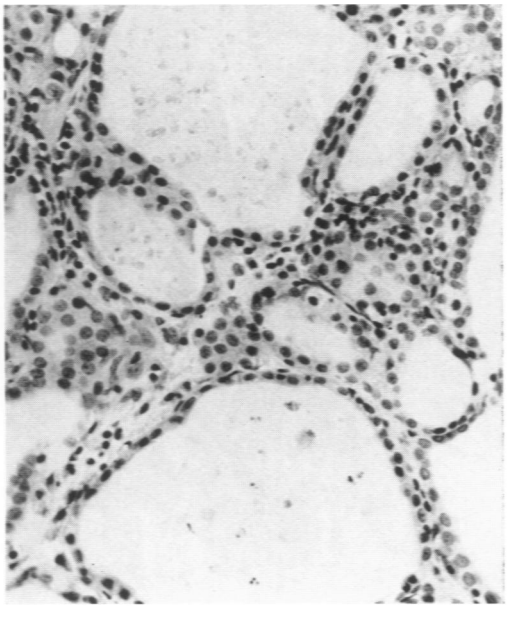

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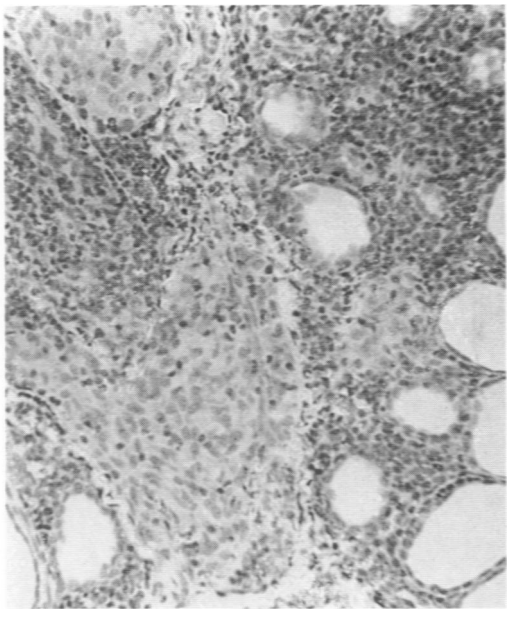
FIG. 9. Rabbit (no. 294) received 2 injections of pooled rabbit thyroid extract and was sacrificed after 3½ months. Antibody titer, 1:325. Note the very dense inflammatory infiltration and proliferating epithelial cells forming solid tubules or nodules. × 96.

FIG. 10. Rabbit (no. 558) received 3 intradermal injections of pooled rabbit thyroid extract and was sacrificed after 7 months. Titer, 1:1280. Interstitial inflammatory infiltration by many eosinophils and some plasma cells. Slight fibrosis may be seen. A distended lymphatic, to the right, contains macrophages and lymphoid cells. × 200.

FIG. 11. Rabbit (no. 678) received one intradermal and numerous intravenous injections of purified hog thyroglobulin and was sacrificed after 5 months. Titer against rabbit thyroid, 1:2187. Several follicles are filled with desquamated epithelial cells, macrophages and leukocytes. There is interstitial infiltration between the preserved follicles which contain unaltered colloid. × 240.

FIG. 12. Guinea pig (no. 88) first received an injection of pooled guinea pig thyroid in the neck region and, 2 months later, in the footpads, and was sacrified after 2½ months. Titer, 1:125. An area of dense inflammation about small follicles is shown. Several disintegrating cells may be seen in adjacent follicles. × 180.



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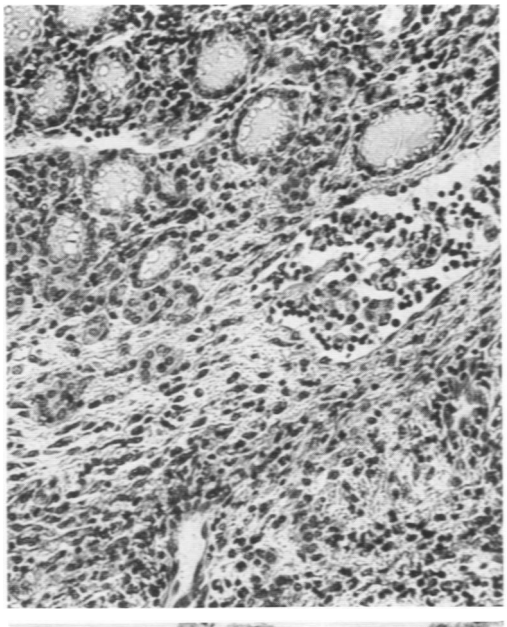

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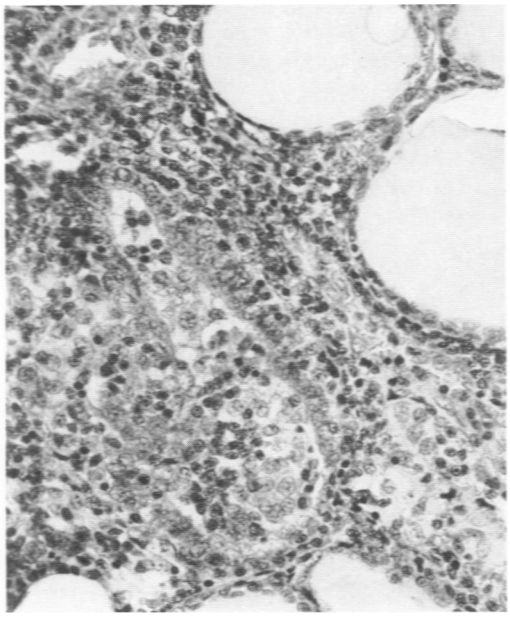

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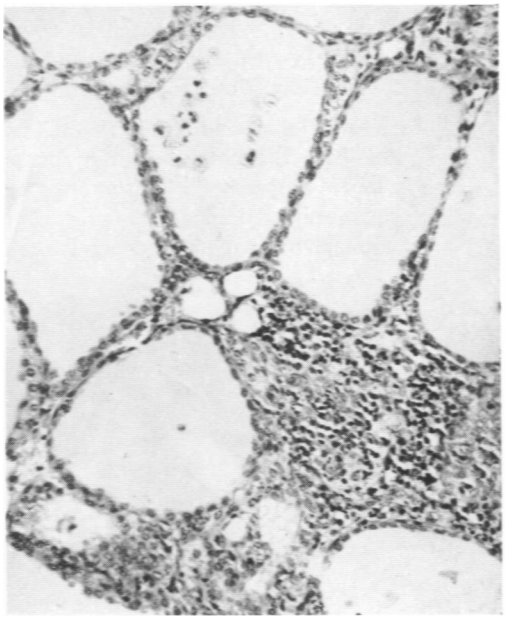

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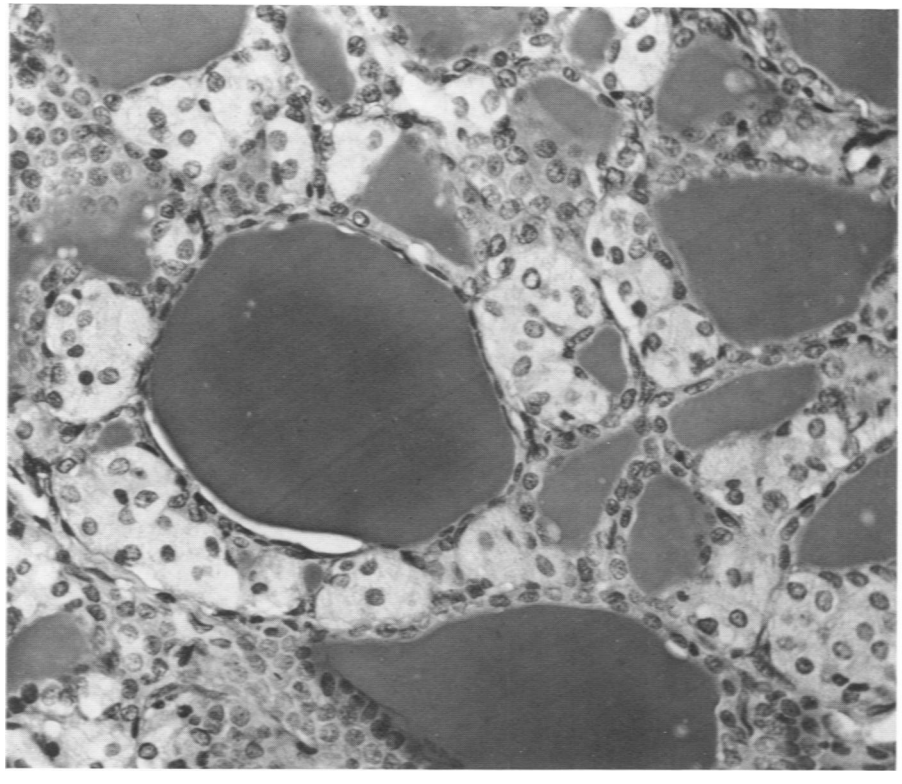
FIG. 13. Rabbit (no. 543) received an extract of human thyroid cancer. A single intradermal and intraperitoneal injection was followed by several intravenous injections. The animal was sacrificed after 4 months. Distinct epithelial swelling with foam cell quality occurs in compact follicles with little or no colloid. Some of the original epithelial cells are undermined and replaced in part by the swollen epithelium. Hotchkiss-McManus stain. × 280.

FIG. 14. Rabbit (no. 539). Material and method of injection as in rabbit no. 543 (Fig. 13). Sacrificed after 3 months. The foamy change is localized within and about the wall of a follicle to the right of the center. Note the disintegration of the epithelial lining in some of the surrounding follicles, with considerable granular and vacuolar debris in their lumens. × 270.



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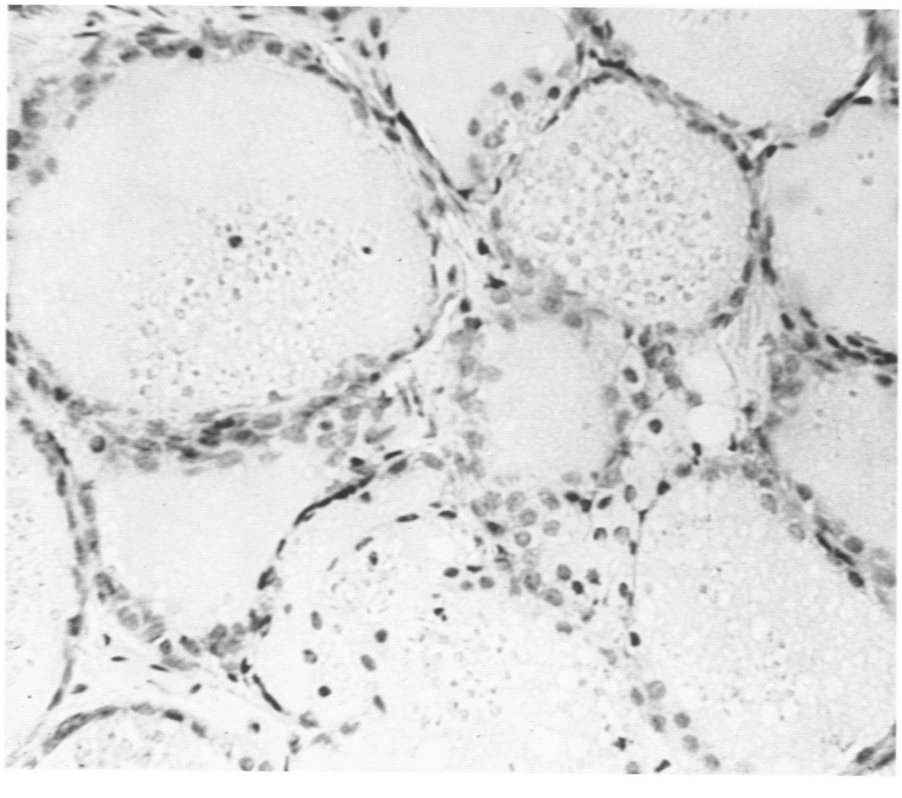

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