vivo effectiveness of this and related compounds are being undertaken in our laboratory.

Summary. *In vitro* preincubation of 1-methyl-3-nitro-1-nitrosoguanidine with *Plasmodium berghei* destroys the infectivity of the plasmodia in mice. None of the structurally similar compounds tested had any effect. Of the anticancer compounds tested, only mitomycin C appears to have an effect. The high level of mitomycin C employed in the experiment (Table III) to obtain the small effect indicates that this compound may not be an effective antimalarial compound *in vivo*. The observations presented in this paper show that 1-methyl-3-nitro-1-nitrosoguanidine represents a promising novel type of antimalarial compound.

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Thyroglobulin-Like Substances in Human Thyroid Subcellular Particulates* (33418)

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In a study of thyroglobulin synthesis, Nunez *et al.* (1) have postulated that thyroglobulin originates from ribosomes of thyroid cells. It is believed that subsequent polymerization and iodination of the precursor protein take place in microsomes and mitochondria. It would be of interest to demonstrate such thyroglobulin precursors in subcellular particulates. This communication presents immunochemical evidence for the presence of thyroglobulin and thyroglobulin-like substance in thyroid mitochondrial and microsomal fractions.

Materials and Methods. Human thyroid glands obtained upon necropsy[1] were homogenized in buffered physiological saline (BPS, 0.16 *M* NaCl in 0.05 *M* phosphate buffer, pH 7.6). The mitochondria were isolated from the homogenate by centrifugation at 10,000 *g* for 10 min after removal of cell debris and nuclei at 1000 *g* for 15 min. The microsomal fraction was obtained by centrifugation for 1 hr at 105,000 *g* from the supernatant after the removal of mitochondria. Both the mitochondrial and microsomal fractions were washed twice with buffered physiological saline. By the treatment of the particulate fractions with sodium deoxycholate in a concentration of 0.5% at pH 7.8, deoxycholate-insoluble fractions were obtained. Cell sap fraction, then, represented the supernatant left over from the original thyroid homogenate after sequential removal of mitochondria and microsomes. Thyroglobulin was fractionated from cell sap by the method of Derrien *et al.* (2) and purified by starch-gel electrophoresis (3). All the fractions were stored in a frozen state until use.

To obtain antisera, thyroid mitochondrial

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[1] Unfortunately we were not able to control the postmortem period, but the thyroid tissue was frozen immediately upon removal from the body.

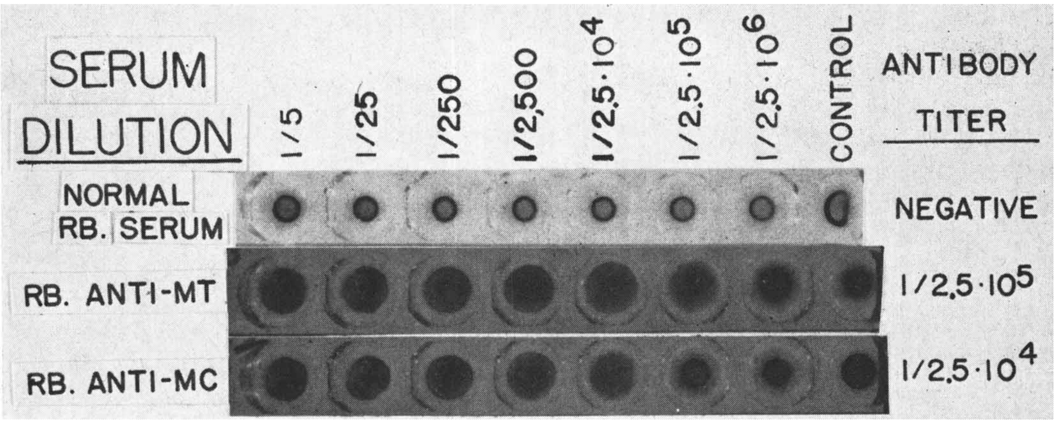

FIG. 1. Hemagglutination test for antibodies (against human thyroglobulin) present in antisera of rabbits (RB) which were previously injected with human thyroid mitochondrial (MT) and microsomal (MC) fractions.

and microsomal fractions, as well as thyroglobulin, were injected into rabbits using adjuvant technique (4). These antisera were allowed to react with various thyroid fractions by the agar double diffusion technique (5) and immunoelectrophoresis (6).

Rabbit antisera directed against human thyroid mitochondria and microsomes were tested for antibody titers against thyroglobulin. A hemagglutination technique was used (7). In this procedure an antiserum was allowed to react with preserved sheep red cells which were previously coated with human thyroglobulin.

Results and Discussion. Rabbit antisera, directed against human thyroid mitochondria and microsomes, were tested for antibody titers against thyroglobulin by the hemagglutination test. These sera show high antibody titers against thyroglobulin (Fig. 1, Table I). The reaction was thyroglobulin-specific as absorption of antisera with thyroglobulin reduced or abolished the titers (Table I).

When the mitochondrial fraction reacted with rabbit antiserum (directed against mitochondria), the agar double diffusion pattern showed 4 precipitin arcs (Fig. 2A). The antiserum also reacted with thyroglobulin and the precipitin arc with thyroglobulin became fused with one of the precipitin arcs of the mitochondrial fraction (Fig. 2A). It was, therefore, indicated that the thyroid mitochondrial fraction (MT) contained thyroglobulin (HTG) or thyroglobulin-like substance. When the mitochondrial fraction reacted with rabbit antiserum against human thyroglobulin (anti-HTG), there were two precipitin arcs (Fig. 2B). One component was identical with 19S thyroglobulin, as the two precipitin arcs became fused together. The other component was antigenically similar to thyroglobulin, but antigenically deficient in that a weak arc appeared separately from the heavy band. The reaction between the deoxycholate-soluble fraction of mitochondria (Sol-MT) and the antiserum against mitochondria (anti-MT) yielded 3 or 4 precipitin arcs, while the residual or deoxycholate-insoluble fraction (Res-MT) did not show any precipitin arc (Fig. 2C). The Sol-MT fraction contained 2 or 3 components which carried thyroglobulin determinants (Fig. 2D).

Immunoelectrophoretic patterns revealed that there were 6 antigenic components for whole mitochondrial fraction (MT) and 3 precipitin arcs from deoxycholate-soluble fraction (Sol-MT) both in reaction with rabbit antiserum against mitochondria (anti-MT) (Fig. 3A). In reaction with antiserum directed against thyroglobulin (anti-HTG), 2 components of the mitochondrial fraction exhibited thyroglobulin antigenicity (Fig. 3B). Furthermore, a precipitin arc resulted when purified thyroglobulin was allowed to react with the rabbit antiserum directed

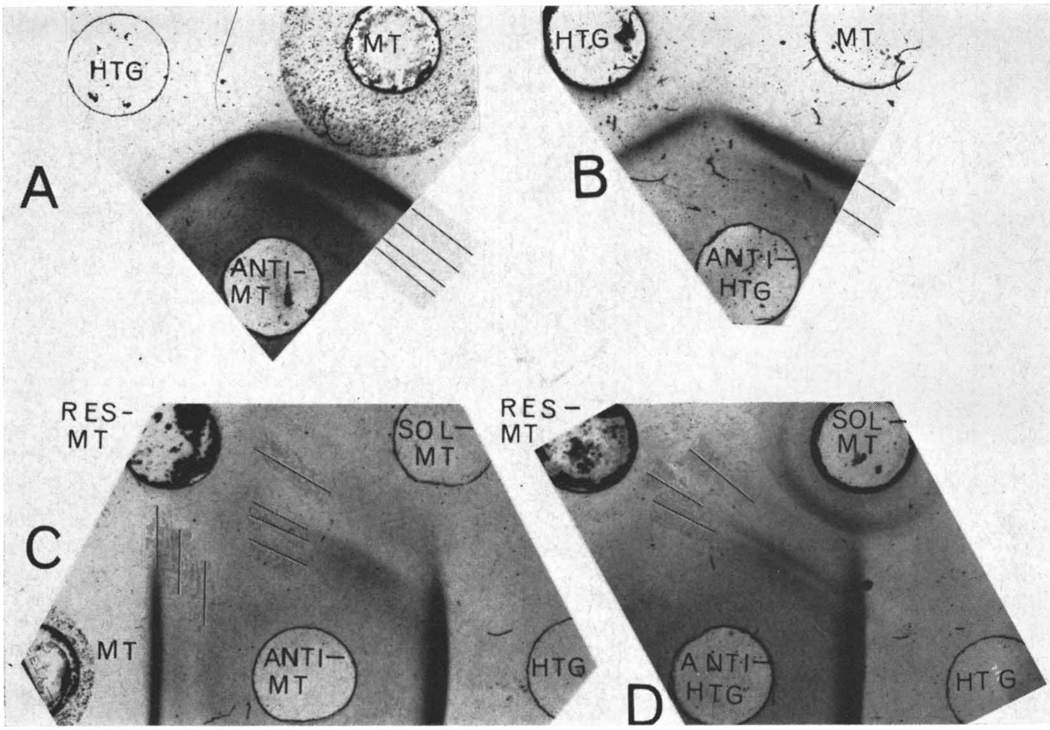

FIG. 2. Immunodiffusion patterns: (A) Thyroid mitochondrial fraction (MT) and human thyroglobulin (HTG) in reaction with rabbit antiserum against MT (anti-MT) (note 4 precipitin arcs from MT). (B) MT and HTG in reaction with rabbit antiserum against thyroglobulin (anti-HTG). Note 2 precipitin arcs between MT and anti-HTG). (C) Thyroid mitochondrial fraction (MT), its subfractions, deoxycholate-soluble fraction (Sol-MT) and deoxycholate-insoluble fraction (Res-MT), as well as HTG in reaction with rabbit antiserum against MT (anti-MT). (D) Same fractions as in (C) reacting with rabbit antiserum against thyroglobulin (anti-HTG).

against the thyroid mitochondrial fraction (Fig. 3C).

Procedures similar to those used to analyze the thyroid mitochondrial fraction were also employed to characterize the thyroid microsomal fraction. An immunodiffusion pattern (Fig. 4A) showed the presence of two antigenic components when the microsomal fraction reacted with rabbit antiserum directed against the microsomal fraction (anti-MC). Deoxycholate-soluble fraction of microsomes (Sol-MC) yielded one precipitin arc whereas deoxycholate-insoluble fraction (Res-MC) did not show any precipitin arc. When thyroglobulin (HTG) was allowed to react with the same antiserum (anti-MC), one precipitin arc was revealed, thus indicating thyroglobulin antigenicity in the original microsomal fraction (Fig. 4A left photo). When rabbit antiserum against thyroglobulin (anti-HTG) was used to react with the microsomal fractions, total microsomal fraction and deoxycholate-soluble microsomal fraction showed one antigenic component, antigenically similar to, but not identical with thyroglobulin (Fig. 4A right photo). In immunoelectrophoretic patterns (Fig. 4B), there were two or three precipitin arcs derived from the microsomal fraction and one or two arcs from Sol-MC fraction in reaction with rabbit antiserum directed against microsomes (anti-MC). When the antigenic materials reacted with an antiserum directed against thyroglobulin (anti-HTG), one precipitin arc was formed both for the microsomes and for the deoxycholate-soluble microsomal fraction. It was apparent that microsomes contained thyroglobulin or thyroblobulin-like substance. This component was dissociable with dexoycholate.

In order to confirm the specificity of the

TABLE I. Hemagglutination Test for Antibodies (against Human Thyroglobulin) in Sera from Patients with Thyroiditis and in Antisera of Rabbits Previously Injected with Human Thyroglobulin, Thyroidal Mitochondrial, and Microsomal Fractions.

Included were antibody titers of the antisera after absorption with various thyroid fractions as indicated.[a]

Antiserum	Absorbed with	Titer (serum dilution)
Human (thyroiditis)	—	$1:2.5 \times 10^4$
	HTG	0
	Thyroid MT	$1:2.5 \times 10^1$
	Thyroid MC	0
Rabbit (vs HTG)	—	$1:2.5 \times 10^6$
	HTG	0
	Thyroid MT	0
	Thyroid MC	0
	Bovine serum alb.	$1:2.5 \times 10^6$
Rabbit (vs thyroid mitochondria)	—	$1:2.5 \times 10^5$
	HTG	0
	Thyroid MT	$1:2.5 \times 10^3$
	Thyroid MC	$1:2.5 \times 10^2$
Rabbit (vs thyroid microsomes)	—	$1:2.5 \times 10^5$
	HTG	0
	Thyroid MT	0
	Thyroid MC	0

[a] HTG = human thyroglobulin; MT = mitochondria; and MC = microsomes.

thyroglobulin antibody, which was found to be present in rabbit antisera directed against thyroid subcellular particulates, radioimmunoelectrophoresis was carried out. Purified human thyroglobulin labeled with ^{131}I was electrophoresed and allowed to react with rabbit antisera directed against thyroglobulin, thyroid mitodhondria, or microsomes. Radioautographs (Fig. 5) showed that it was, indeed, thyroglobulin with which the antisera reacted.

Our data present direct immunochemical evidence for the presence of thyroglobulin and thyroglobulin-like substance in the thyroid subcellular particulates. Since the particulates were prepared by differential centrifugation and repeated washings with physiological saline, it seems unlikely that the presence of these substances results from contamination with the supernatant. Moreover, these thyroglobulin-like substances were dissociable upon the treatment with deoxycholate. Our findings, therefore, support the view of Nunez *et al.* (1), that thyroid mitochondria and microsomes are involved in the biosynthetic pathways of thyroglobulin. The component, antigenically similar to thyroglobulin, but antigenically deficient as compared to 19S thyroglobulin, is of interest. It has a molecular size smaller than 19S thyroglobulin as inferred by gel filtration. That component may indeed correspond to 7S and 12S thyroglobulin subunits as observed by Seed and Goldberg (8).

Our results have shown that thyroid subcellular particulates contain two or more components antigenically similar to thyroglobulin. These antigens elicit antibody

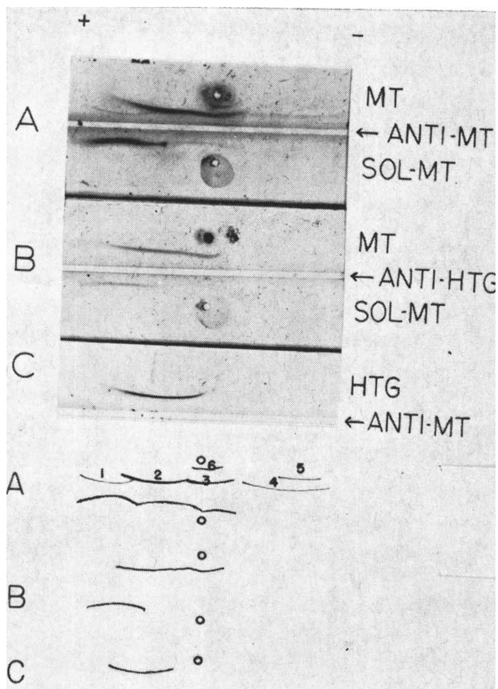

FIG. 3. Immunoelectrophoretic patterns: (A, top well), thyroid mitochondrial fraction (MT); (bottom well), deoxycholate-soluble thyroid mitrochondrial fraction (Sol-MT); trough: rabbit antiserum against MT. (B, top well), same as 3A; (bottom well), same as 3A; trough: rabbit antiserum against human thyroglobulin (HTG). (C) well: human thyroglobulin (HTG); trough: rabbit antiserum against MT.

formation against thyroglobulin. Thyroglobulin molecules bound to subcellular particulates are, therefore, immunogenically comparable to soluble or unbound thyroblobulin. It seems reasonable to consider the possibility that antibodies to thyroid tissue seen in the "auto-immune" reaction might result from the sensitization with particulate-bound thyroglobulin antigens as well as from other possible mechanisms.

Summary. Human thyroid subcellular fractions, mitochondrial and microsomal, and soluble thyroglobulin were separated from thyroid glands and injected into rabbits. Rabbit antisera obtained were tested for antibodies against thyroglobulin. By hemmagglutination tests, antisera directed against thyroid subcellular particulates showed high titers against thyroglobulin. Immunodiffusion and immunoelectrophoresis experiments with thyroid subcellular particulates revealed the presence of several antigenic components; one was identical with thyroglobulin and one or two others were similar to, but antigenically deficient as compared to thyroglobulin. These thyroglobulin-like substances were bound to subcellular particulates, but dissociable upon the treatment with deoxycholate.

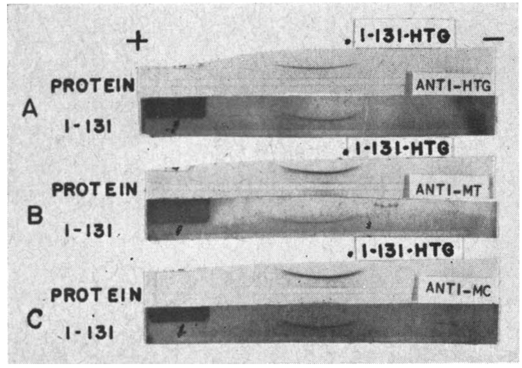

FIG. 5. Radio-immunoelectrophoresis of ^{131}I-human thyroglobulin (HTG^{131}I) reacting with rabbit antisera against (A) HTG, (B) human thyroid mitochondria (MT), or (C) human thyroid microsomes (MC). Slides after immunoelectrophoresis were stained with amido schwarz for proteins (top photo) and exposed to photographic film in obtaining radioautographs (bottom photo).

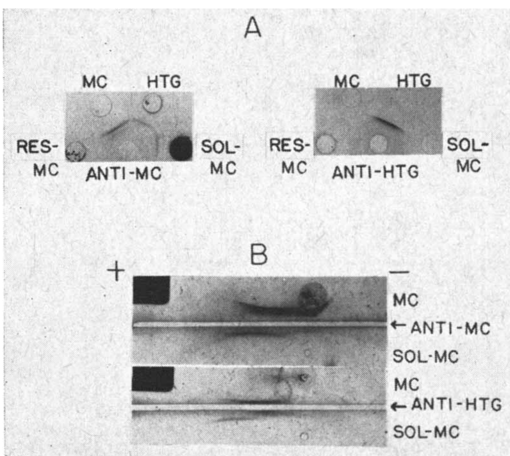

FIG. 4. Immunodiffusion patterns: left photo—human thyroglobulin (HTG), thyroid microsomal fraction (MC), deoxycholate-soluble fraction of MC (Sol-MC), and deoxycholate-insoluble fraction of MC (Res-MC) in reaction with rabbit antiserum against MC (anti-MC); right photo—same fractions in reaction with rabbit antiserum against HTG (anti-HTG). (B) Immunoelectrophoretic patterns: same designations as in (A).

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