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Biochemical Mechanisms of the Antileishmanial Activity of Sodium Stibogluconate

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Pentavalent antimonial agents such as sodium stibogluconate (Pentostam; Burroughs Wellcome Co., London, United Kingdom) are the drugs of choice for the treatment of leishmaniasis, but their biochemical mechanisms of action are virtually unknown. The viability of *Leishmania mexicana* (WR 227) promastigotes and amastigotes was decreased 40 to 61% by a 4-h exposure to 500 μg of Sb (in the form of stibogluconate) per ml. Such exposure also resulted in a 51 to 65% decrease in incorporation of label into DNA, RNA, and protein; a 56 to 65% decrease in incorporation of label into purine nucleoside triphosphate; and a 34 to 60% increase in incorporation of label into purine nucleoside monophosphate and diphosphate. It is postulated that the apparent decrease in ATP and GTP synthesis from ADP and GDP contributes to decreased macromolecular synthesis and to decreased *Leishmania* viability. Further experiments suggested that inhibition of glycolysis and the citric acid cycle may partially explain the inability to phosphorylate ADP.

Leishmaniasis is initiated when sand flies inject the extracellular promastigote form of the parasite into the skin. Promastigotes are rapidly phagocytized, after which they transform into the intracellular amastigote form. Promastigotes are not seen in clinical lesions; the multiplication of amastigotes within macrophages leads to clinical disease.

Pentavalent antimonial agents in the form of sodium stibogluconate (Pentostam; Burroughs Wellcome Co., London, United Kingdom) or meglumine antimonate (Glucantime; Specia, Paris, France) are the primary therapeutic agents for all forms of leishmaniasis (1, 10). Although some form of antimony may have been in use for leishmaniasis since the days of the ancient Greeks (16) and the present formulations were developed in the 1940s, the biochemical mechanisms of the antileishmanial activity of antimony are virtually uninvestigated.

Until recently, trivalent antimonial agents were used clinically for schistosomiasis. Bueding and Mansour found that 10^{-4} to 10^{-3} M (approximately 30 to 300 μg/ml) potassium antimony tartrate inhibited *Schistosoma mansoni* survival and schistosomal phosphofructokinase activity by 50% but poorly inhibited mammalian phosphofructokinase (3, 11). Since the product of the phosphofructokinase reaction is the substrate for aldolase and aldolase is the rate-limiting enzyme in *S. mansoni* glycolysis, the authors reasoned that the mechanisms by which trivalent Sb inhibited schistosomal glycolysis had been elucidated. They also hypothesized that such inhibition of glycolysis might be the mechanism for the antischistosomal activity of the drug and for the greater toxicity of Sb to schistosoma than to mammalian cells.

We are not aware of a systematic evaluation of the effect of antimonial agents on leishmanial energy metabolism or on other leishmanial biochemical pathways. We report here the effect of antileishmanial concentrations of stibogluconate on macromolecular synthesis, purine nucleotide synthesis, and energy metabolism of *Leishmania mexicana* promastigotes and amastigotes.

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MATERIALS AND METHODS

***Leishmania* strain.** The strain used in these experiments, *L. mexicana* WR 227, was originally cultured from a patient who acquired a cutaneous infection in Panama. The lesions were not cured with standard dosages of sodium stibogluconate, possibly because the immune response of the patient to *Leishmania* antigen was borderline. In a standard in vitro test in which macrophages containing amastigotes are exposed to drug for 6 days, WR 227 amastigotes in human macrophages were more susceptible to stibogluconate than all four other strains of amastigotes tested (2).

In the present experiments, *L. mexicana* promastigotes were maintained in Schneider Drosophila medium (GIBCO Laboratories, Grand Island, N.Y.) revised with the addition of 20% heat-inactivated fetal calf serum (8). Promastigotes were grown to late-log phase (40×10^6/ml) and suspended in fresh medium at a concentration of 30×10^6 to 40×10^6/ml for experimentation.

L. mexicana amastigotes were obtained from infected J774 macrophages by the method of Chang (4). In brief, J774 macrophages (American Type Culture Collection, Rockville, Md.) in medium consisting of RPMI 1640 (GIBCO) containing 15% fetal calf serum were grown to confluence on one surface of plastic culture flasks (model 3150; Costar, Cambridge, Mass.) at 34.5°C. The approximately 30×10^6 macrophages per flask were infected with a 30-fold multiplicity of *L. mexicana* WR 227 promastigotes, previously grown to stationary phase (50×10^6/ml [15]). After 18 h and on each subsequent day, the culture medium was replaced with an equal volume of fresh medium. By 3 days after infection, there were no free promastigotes, virtually all macrophages contained amastigotes, and there was a range of 25 to 30 amastigotes per macrophage. Amastigotes were freed from their macrophage hosts by passing the infected macrophages twice through a 30-gauge needle. Freed amastigotes were purified from other subcellular fractions and from unbroken macrophages by suspension of the disrupted macrophage cultures in 45% Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) and by centrifugation of this suspension onto

100% Percoll at 3,500 × g for 30 min (4). The 45 to 100% Percoll interface containing the amastigotes was diluted with 30 volumes of physiologic saline and centrifuged at 3,500 × g for 15 min. The amastigote pellet was again washed at 3,500 × g for 15 min. Approximately 80% of the amastigotes originally within macrophages were recovered after the 2-h isolation procedure. Electron microscopic observation revealed that the organisms were intact and pure except for the occasional presence of host membrane fragments. Amastigotes were suspended at a concentration of 30 × 10^6 to 40 × 10^6/ml of RPMI 1640 plus 10% fetal calf serum for experimentation.

Antileishmanial activity. To determine the antileishmanial activity of stibogluconate over the 4-h period during which biochemical experiments were conducted, 1 ml of promastigotes or amastigotes in their respective media was exposed to 0 to 500 μg of Sb in the form of sodium stibogluconate for 4 h at 24°C (promastigotes) or 34.5°C (amastigotes). After being washed twice (1,000 × g for 10 min) to remove excess drug, the organisms were suspended at a concentration of 10^6/ml of promastigote culture medium and incubated for 2 days at 24°C. The number of promastigotes per milliliter was determined on each of the 2 days in each culture.

Biochemical experiments. To determine incorporation of precursors into *Leishmania* macromolecules, 1 ml of organisms was simultaneously exposed to 0 to 500 μg of Sb and to 0.4 μCi of [^{14}C]leucine (333 mCi/mM; New England Nuclear Corp., Boston, Mass.), 1 μCi of [^{3}H]uridine (20 mCi/mM; New England Nuclear), or 1 μCi of [^{3}H]thymidine (20 mCi/mM; New England Nuclear). After 2 or 4 h, the organisms were centrifuged at 5,000 × g for 30 s in a microfuge, suspended in 0.5 ml of cold phosphate-buffered saline, and added to 0.5 ml of cold 0.6 N trichloroacetic acid (TCA). After 30 min, the TCA-precipitated material was washed on filter paper with 10 volumes of water and 10 volumes of methanol. The filter paper was dried and counted in a scintillation counter.

To determine synthesis of purine nucleotides in Sb-exposed organisms, 1 ml of organisms was simultaneously exposed to 0 to 500 μg of Sb and to 5 μCi of [^{3}H]hypoxanthine (10 mCi/mM; New England Nuclear) for 4 h, after which the organisms were pelleted and exposed to TCA as detailed above. The acidic supernatant was extracted and brought to pH 5.0 to 5.5 with trioctylamine-Freon by the method of Pogolotti and Santi (13). A portion (2.5 μl) of the 100-μl trioctylamine-Freon extract that contained the nucleotides was spotted onto polyethyleneimine-cellulose thin-layer chromatographic plates (Brinkmann Instruments, Inc., Westbury, N.Y.), overspotted with a combination of AMP, ADP, and ATP, and eluted for 2 h with 1.0 M LiCl as solvent (14). The spots visualized with UV light corresponding to the nucleotide standards were cut out and counted in a scintillation counter. One sample was also analyzed by high-pressure liquid chromatography (HPLC) by the procedure of Pogolotti and Santi (13).

To determine CO_2 formation from glucose or acetate in Sb-exposed *Leishmania*, 1 ml of organisms was exposed to 0 to 500 μg of Sb for 4 h. After the organisms were pelleted, they were incubated with 2 μCi of [^{14}C]glucose (55 mCi/mM; New England Nuclear) per ml or with 1 μCi of [^{14}C]acetate (2 mCi/mM; New England Nuclear) per ml for 0.5 h under conditions such that CO_2 formed by the parasites would be trapped in $Ba(OH)_2$-coated filter paper (5). The dried filter paper was then counted in a gas flow detector (5).

Transport of [^{14}C]2-deoxy-D-glucose (nanomoles of uptake per grams of protein per 5 s) or of [^{14}C]acetate by promastigotes or by amastigotes was determined in organisms by methods previously described (6).

The incorporation of [^{14}C]hypoxanthine into J774A1 macromolecules or into the nucleotides of J774A1 cells was determined by exposing J774A1 macrophages (American Type Culture Collection), at a concentration of 10 × 10^6 cells per ml of RPMI 1640-10% heat-inactivated fetal calf serum, simultaneously to 0 to 500 μg of Sb per ml and to 5 μCi of [^{14}C]hypoxanthine per ml for 4 h, after which the incorporation of label into the TCA pellet and into the TCA supernatant was determined as detailed above.

RESULTS

Antileishmanial activity of stibogluconate. To determine appropriate stibogluconate concentrations for biochemical experiments, promastigotes and amastigotes were exposed to 0 (controls) to 500 μg of Sb per ml for 4 h. There was no alteration of organism number or of their light-microscopic morphology during this time period. Such exposure, however, resulted in decreased viability of the organisms during the subsequent 2 days. Exposure of promastigotes to 500 μg of Sb per ml for 4 h resulted in 40% inhibition in the ability of the organisms to multiply during that 2-day period (Table 1). The effect of Sb on amastigotes was more complex. Amastigotes exposed to all Sb dosages demonstrated an impaired ability to transform into and multiply as promastigotes during the following day. This inhibition was partially reversible, since the number of promastigotes in these cultures was much closer to the control number on day 2 after drug exposure than on day 1. Sb dosages of 150 to 500 μg per ml were used in biochemical experiments so that the biochemical effect of both slightly and highly antileishmanial Sb concentrations would be assessed.

Inhibition of *Leishmania* macromolecular synthesis by stibogluconate. Stibogluconate-induced inhibition of incorporation of radiolabeled leucine, uridine, and thymidine into leishmanial protein, RNA, and DNA over 4 h is shown in Table 2. For both promastigotes and amastigotes, there was a 51 to 65% inhibition of protein, RNA, and DNA synthesis in organisms exposed to 500 μg of Sb per ml. The inhibition of macromolecular synthesis was time dependent: at 2 h, there was 30 to 40% inhibition of macromolecular synthesis in organisms exposed to 500 μg of Sb per ml (data not shown).

Inhibition of leishmanial purine nucleotide formation by stibogluconate. Because inhibition of protein synthesis could

TABLE 1. Antileishmanial activity of a 4-h exposure to stibogluconate[a]

Sb treatment (μg/ml)	% Control promastigotes in cultures derived from Sb-exposed[b]:			
	Promastigotes on day:		Amastigotes on day:	
	1	2		
150	94 (83–105)	90 (71–110)	65 (42–89)	75 (70–79)
300	73 (56–90)	74 (60–88)	7 (0–13)	62 (48–87)
500	56 (53–60)	60 (54–66)	1 (0–3)	39 (36–43)
0 (control)[c]	3 × 10^6	20 × 10^6	2.5 × 10^6	20 × 10^6

[a] Promastigotes or amastigotes were exposed to 0 (controls) to 500 μg of Sb (as stibogluconate) per ml for 4 h, then washed, and suspended in promastigote medium. The number of promastigotes per ml was counted 1 and 2 days later.

[b] Data represent the number of promastigotes per milliliter in experimental groups expressed as a percentage of the number in simultaneously cultivated controls (mean of two experiments [range]).

[c] The mean value of control data is shown as the number of promastigotes per milliliter.

TABLE 2. Inhibition of *Leishmania* protein, RNA, and DNA synthesis by stibogluconate[a]

Sb treatment (μg/ml)	% Control cpm in[b]:					
	Promastigote			Amastigote		
	Protein	RNA	DNA	Protein	RNA	DNA
150	83 (76–90)	77 (65–90)	62 (50–74)	80 (72–88)	90 (81–100)	87 (69–104)
300				53 (52–54)	60 (55–65)	75 (70–80)
500	47 (45–50)	35 (22–47)	41 (39–44)	42 (32–52)	40 (32–49)	49 (38–60)
0 (control)[c]	8,407	3,039	6,005	8,890	5,853	2,929

[a] One milliliter of promastigotes or amastigotes (20×10^6) was exposed to Sb and to [^{14}C]leucine, [^{3}H]uridine, or [^{3}H]thymidine for 4 h, after which the TCA-precipitable material was counted.
[b] Data represent counts per minute in Sb-exposed organisms expressed as a percentage of simultaneously cultivated controls (mean of two experiments [range]).
[c] The mean value of control data (counts per minute) is also shown.

result from inhibition of nucleic acid synthesis and because the latter could result from depletion of nucleoside triphosphates, purine nucleotide formation in stibogluconate-treated *L. mexicana* was determined.

Control promastigotes and amastigotes exposed to label for 4 h demonstrated nucleoside triphosphate-to-diphosphate (TP/DP) ratios of 6.3 and 7.2, respectively (Table 3). In promastigotes exposed to 500 μg of Sb, the incorporation of label into nucleoside triphosphates decreased by 65% (Table 3). In contrast, the incorporation of label into nucleoside monophosphates and diphosphates increased by 34 to 60% (Table 3). As a result, the TP/DP ratio decreased by a factor of 2, from 6.3 in controls to 3.1. The incorporation of label into amastigotes exposed to 500 μg of Sb per ml similarly demonstrated a 56% decrease in nucleoside triphosphate and a 44 to 60% increase in nucleoside monophosphates and diphosphates. The TP/DP ratio decreased by a factor of 3.6, from 7.2 in controls to 2.0 (Table 3).

To verify that thin-layer chromatographic procedures accurately determined the number of nucleotides, the number of nucleotides in one experiment in which amastigotes were exposed to Sb for 4 h was also determined by HPLC procedures. The HPLC-determined ratio of radiolabeled TP/DP (Table 4) closely paralleled the values determined by thin-layer chromatography. For this experiment, the TP/DP values determined by thin-layer chromatography were: control, 8.1; 150 μg of Sb per ml, 7.2; 300 μg of Sb per ml, 3.9; and 500 μg of Sb per ml, 1.9. Because HPLC determines adenosine and guanosine nucleotides separately, in this experiment it was found that the decrease in the radiolabeled TP/DP ratio was due to decreased nucleoside triphosphate labeling and increased nucleoside monophosphate and diphosphate labeling for both adenosine and guanosine nucleotides. Nucleotide pools were also determined by HPLC. The ratio of total purine triphosphates to diphosphates was 6.7 in controls and 2.7 in organisms exposed to 500 μg of Sb per ml. These data indicate that the turnover in purine nucleotides was sufficiently rapid such that apparent inhibition of trinucleotide synthesis during 4 h resulted in inhibition of total purine trinucleotides.

Inhibition of *Leishmania* energy metabolism by stibogluconate. Because inhibition of phosphorylation of ADP could result from decreased glycolysis and citric acid cycle activity, the effect of Sb treatment on *Leishmania* CO_2 formation from radiolabeled glucose and acetate was determined. Drug-treated promastigotes and amastigotes demonstrated a dose-dependent inhibition of CO_2 formation from both glucose and acetate (Table 5). The inhibition of CO_2 formation from glucose was greater than the inhibition of CO_2 formation from acetate for both forms of the organism. The difference between inhibition of CO_2 formation from glucose versus that from acetate was slight for organisms exposed to 150 or 300 μg of Sb per ml but was marked for organisms exposed to 500 μg of Sb per ml. Table 5 also demonstrates that the inhibition of catabolism of glucose and of acetate was greater in promastigotes than in amastigotes.

To verify that decreased glucose and acetate catabolism was not due to decreased membrane transport by drug-exposed organisms, the transport of radiolabeled deoxyglucose and acetate was determined under several conditions. There was less than a 2% decrease in the velocity of deoxyglucose and acetate transport (nanomoles of uptake per grams of protein per 5 s) for both promastigotes and amastigotes exposed to 150 to 500 μg of Sb per ml when the precursor

TABLE 3. Inhibition of *Leishmania* purine trinucleotide formation by stibogluconate[a]

Organism	Sb treatment (μg/ml)	% Control cpm in:			TP/DP
		MP	DP	TP	
Promastigotes	150	115 (91–140)	98 (83–113)	70 (62–79)	4.8 (3.4–6.2)
	300	120 (115–124)	92 (92–93)	55 (55–56)	4.2 (3.9–4.6)
	500	160 (145–175)	134 (73–196)	35 (35–36)	3.1 (3.1–3.1)
	0 (control)	322	938	5,926	6.3
Amastigotes	150	99 (92–106)	115 (105–126)	105 (96–114)	6.4 (5.5–7.2)
	300	150 (148–151)	148 (129–167)	100 (84–115)	5.3 (3.9–6.7)
	500	160 (107–213)	144 (142–146)	44 (36–52)	2.0 (1.9–2.1)
	0 (control)	117	273	1,960	7.2

[a] One milliliter of promastigotes or amastigotes (30×10^6) exposed to 0 to 500 μg of Sb and to [^{14}C]hypoxanthine for 4 h were TCA treated, and 2.5 μl of the trioctylamine-Freon extract of the TCA supernatant was chromatographed on thin-layer plates. Spots corresponding to nucleoside monophosphates (MP), diphosphates (DP), and triphosphates (TP) were cut out and counted. Data represent counts per minute in Sb-exposed organisms expressed as a percentage of control counts (mean of two experiments [range]). Mean control values (counts per minute) are also listed.

TABLE 4. Inhibition of amastigote adenosine and guanosine nucleotide formation by stibogluconate[a]

Sb treatment (μg/ml)	% Control cpm in:						ATP/ ADP	GTP/ GDP	TP/DP
	AMP	ADP	ATP	GMP	GDP	GTP			
150	135	124	100	110	100	100	7.1	4.5	6.6
300	255	201	67	151	136	60	2.9	1.7	2.8
500	489	150	26	394	80	21	1.5	1.0	1.5
0 (control)	2,815	21,107	185,161	587	4,534	17,847	8.8	3.9	7.9

[a] One hundred microliters of the trioctylamine-Freon supernatants of one of the experiments in Table 3 in which amastigotes were exposed to Sb for 4 h was analyzed by the HPLC procedures of Pogolotti and Santi (13).

was added after 2 min of preincubation of organisms with drug. There were 37 and 20% decreases in deoxyglucose and acetate transport, respectively, for promastigotes preincubated with 500 μg of Sb per ml for 4 h and 38 and 14% decrease in deoxyglucose and acetate transport, respectively, in similarly exposed amastigotes. Comparison of the decrease in transport after 4 h of drug exposure with the decrease after 5 s suggests that the decreased transport after 4 h was due to generalized disturbance of *Leishmania* function rather than to decreased transport per se.

Inhibition of J774 macrophage metabolism by stibogluconate. To determine whether stibogluconate inhibited incorporation of nucleic acids and nucleotides in mammalian cells to an extent comparable to that in *L. mexicana*, the effect of a 4-h stibogluconate exposure on incorporation of radiolabeled hypoxanthine into nucleic acids and nucleotides of the mammalian macrophage J774 cell line was examined. In two experiments, there was a slight, dose-dependent decrease in the incorporation of label into nucleic acids such that exposure to 500 μg of Sb per ml resulted in a mean of 77% of the incorporation of label into control nucleic acids. The TP/DP ratio was measured for one of these experiments. The ratio was 5.1 in controls and did not decrease in cells exposed to 500 μg of Sb per ml (TP/DP, 6.5).

DISCUSSION

The mechanism of trivalent antimonial agents against schistosomes was hypothesized to involve inhibition of energy production via inhibition of the glycolytic enzyme phosphofructokinase. The mechanisms of pentavalent antimonial agents against *Leishmania* species, for which the drugs are still the primary chemotherapeutic agent, have apparently not been the subject of detailed investigation.

In a standard in vitro model in which amastigotes within human macrophages are exposed to drug for 6 days, 77 to 91% of *L. mexicana* WR 227 amastigotes are eliminated by exposure to 20 μg of Sb per ml, the approximate peak human serum level after stibogluconate treatment (2). Because biochemical experiments are generally performed with isolated organisms over shorter time periods, free-living promastigotes and pure amastigotes isolated from an infected tumor cell model were exposed for 4 h to higher concentrations of Sb. A 4-h exposure to 500 μg of Sb per ml resulted in sufficient biochemical damage such that only 1% of the amastigotes were capable of transformation to promastigotes over the next 18 h. The impairment in amastigotes was partially reversible. On the next day, the relative number of promastigotes derived from Sb-exposed (500 μg/ml) amastigotes (39% of controls) was only somewhat less than the relative number of promastigotes derived from Sb-exposed promastigotes (60% of controls).

Protein, RNA, and DNA syntheses were decreased in a dose-dependent manner in Sb-exposed *L. mexicana*, such that macromolecular synthesis was 35 to 49% of controls in both promastigotes and amastigotes exposed to 500 μg of Sb per ml for 4 h. There was a concomitant dose-dependent specific decrease in net purine nucleoside triphosphate formation from diphosphates. In organisms exposed to 500 μg of Sb per ml, ATP plus GTP levels were 35 to 44% of those of controls, and nucleoside monophosphate and diphosphate levels were 134 to 160% of those of controls. We hypothesize that the decreased net formation of purine nucleoside triphosphates contributed to the decrease in macromolecular synthesis, which in turn contributed to the decreased viability of organisms exposed to Sb in vitro. The more modest decrease in formation of nucleic acid and nucleoside triphosphates in the mammalian cell line (J774) suggests that the increased toxicity of stibogluconate to *L. mexicana* compared with that to mammalian cells may be related to these biochemical mechanisms.

In mammalian cells, purine nucleoside diphosphates may be phosphorylated by cytoplasmic kinases, but the major site of phosphorylation of ADP to ATP is in the mitochondrion as NADH generated by glycolysis and the citric acid cycle is utilized. The overall activity of glycolytic, hexose monophosphate and citric acid enzymes may be assessed by determining CO_2 formation from glucose. The activity of citric acid cycle enzymes alone can be assessed by determining CO_2 formation from acetate.

If it is assumed that *Leishmania* promastigote energy metabolism generally parallels that in mammalian cells, the demonstration that CO_2 generation from glucose was inhibited to a greater extent than CO_2 generation from acetate in Sb-exposed promastigotes indicates that both glycolysis-plus-hexose monophosphate activity and the citric acid

TABLE 5. Inhibition of *Leishmania* CO_2 production from glucose and acetate by stibogluconate[a]

Organism	Sb treatment (μg/ml)	% Control CO_2 formation from[b]:	
		Glucose	Acetate
Promastigotes	150	70 (64–76)	74 (73–76)
	300	35 (27–43)	46 (42–51)
	500	10 (9–11)	38 (33–42)
	0 (control)	3,400	500
Amastigotes	150	89 (85–93)	93 (87–100)
	300	75 (75–76)	76
	500	56 (50–62)	77 (68–86)
	0 (control)	1,900	423

[a] One milliliter of promastigotes or amastigotes (30×10^6) was incubated with Sb for 4 h and then exposed to [^{14}C]glucose or [^{14}C]acetate for 0.5 h under conditions such that $^{14}CO_2$ was trapped and counted.

[b] Data represent amount of CO_2 in Sb-exposed organisms expressed as a percentage of controls (mean of two experiments [range]). Control values (counts per minute per 10^6 organisms) are also shown.

cycle were inhibited by Sb. It is therefore unlikely that the glycolytic enzyme phosphofructokinase is the sole enzyme in these pathways that is affected by Sb, as has been postulated for Sb-treated schistosomes. However, the lack of detailed knowledge of *Leishmania* energy metabolism makes it difficult to correlate the inhibition of glucose and acetate catabolism with the inhibited ability of Sb-exposed promastigotes to phosphorylate ADP. For example, the contribution of glucose catabolism to promastigote energy requirements has been questioned (7, 12), and the relative importance of glycolysis (which generates NADH) and the hexose monophosphate shunt (which generates NADPH) is unknown (12). In amastigotes, inhibition of CO_2 formation from glucose and acetate catabolism was modest and approximated the decrease in transport, so that the effect of Sb on catabolism of glucose and acetate may have been slight.

Inhibition of purine nucleotide phosphorylation may involve inhibition by Sb of other cytoplasmic and mitochondrial enzymes in addition to Sb-induced inhibition of glucose metabolism and of the citric acid cycle. We have recently demonstrated that the primary fine-structural change in macrophage-contained amastigotes exposed to Sb is lack of definition of mitochondrial and other internal membranes (9). The electron microscopic findings suggest that mitochondrial oxidative phosphorylation may be a target of Sb activity.

Although only certain biochemical pathways were examined in these experiments, the profound inhibition of purine nucleoside triphosphate and macromolecular synthesis in amastigotes exposed to in vitro *L. mexicana*-suppressive concentrations of stibogluconate suggests that the leishmanicidal activity of pentavalent antimonial agents may result from such biochemical events.

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