testing of a larger number of isolates of LSc2ab after human passage, especially virus isolated 3 and 4 weeks after administration of the LSc2ab vaccine. The 4 fecal strains of LSc2ab available for testing were all inhibited by the 2 markers, but isolates containing high titers of virus would be needed to determine the limits of the inhibitory effect.

It seems reasonable to conclude even after testing only 17 strains that wild strains sensitive to this combination of inhibitors are rare. This tentative conclusion gains support from the unselected nature of the wild strains tested — all except one only slightly if at all effected by the inhibitors — and from published information which indicates that most strains of wild type 1 virus are not inhibited by even one of the inhibitors used alone(5,11). The one wild strain that was partially inhibited by the equine serum and dextran sulfate was still distinguishable from LSc2ab. In such cases graded concentrations of equine serum and dextran sulfate could be tried since the range of concentrations of serum inhibited LSc2ab is large, whereas the range affecting even partly sensitive wild virus is apt to be much narrower.

Summary. The antiviral properties of two substances, dextran sulfate and equine serum, were made use of in combination to distinguish the LSc2ab (Sabin) strain of type 1 poliovirus from other type 1 strains. The combination of inhibitors strongly suppressed plaque formation of LSc2ab virus. In plaque reduction tests with the combined inhibitors 17 or 18 strains of type 1 poliovirus, including attenuated, wild and virulent strains, were unaffected, and these were readily distinguished from the LSc2ab virus.

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Selective Inhibition of Deoxyribonucleic Acid Synthesis by Salicylhydroxamic Acid.* (31369)

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Previous communications regarding hydroxyurea and other hydroxamic acids have described various pharmacological actions of compounds of this type(1-7); consequently a number of other hydroxamic acids are being synthesized and investigated regarding antimicrobial and antitumor properties, effects on the central nervous system, and mode of metabolic alteration.

In the course of testing several such compounds to determine effects on deoxyribonucleic acid synthesis *in vitro,* salicylhydroxamic acid was noted to exert a selective effect on the test system; a report follows.

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Materials and methods. Salicylhydroxamic acid (SHA) was obtained from Hynes Chemical Research Corp., Durham, N. C., and was recrystallized from methyl alcohol by addition of water followed by cooling to 5°C for 15 hours. Radioisotopes were from New England Nuclear Corp., and NCTC-109 medium was from Microbiological Associates. Other chemicals were of reagent grade from various commercial sources. The Ehrlich ascites tumor strain was originally obtained from the Department of Zoology, Duke University, and has been propagated in Swiss Webster mice for approximately 3 years.

Tumor cells were removed from mice 5 to 12 days after inoculation and were washed twice with a mixture of NCTC-109 medium (8 parts) and 0.1 M Na-K phosphate buffer, pH 7.4 (2 parts); all subsequent manipulations of the cells were done in this suspending medium. Twenty mm outside diameter test tubes served as reaction vessels and were maintained at 37°C with gentle agitation. Each reaction mixture consisted of 5 ml of a 1% (v/v) cell suspension, 0.025 ml dimethylsulfoxide with or without the test compound, and 0.5 ml of 0.9%NaCl containing the appropriate isotope. This latter volume contained thymidine-^{3}H (1 or 10 μc), uridine-^{3}H (1 μc), or uniformly labeled L-leucine-^{14}C (1 μc) to measure synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein, respectively. Two ml samples were removed at the indicated times and added to 2 ml of 10% trichloroacetic acid (TCA) at 0°C. The insoluble precipitate was washed with 5% TCA three times by sedimentation and resuspension, and finally suspended in 0.5 ml methyl alcohol. Two ml hydroxide of Hyamine (Packard Instrument Co.) was added to effect solubilization, and the contents of each tube were transferred quantitatively to 15 ml of phosphor solution (PPO-POPOP, Packard) in toluene. Radioactivity of each sample was measured with a Packard Tri-Carb liquid scintillation spectrometer.

Results. A typical dose-response relationship of DNA synthesis by ascites tumor cells to increasing concentrations of SHA is shown in Fig. 1. The concentration conferring 50% inhibition was just under 4×10^{-4} M; this

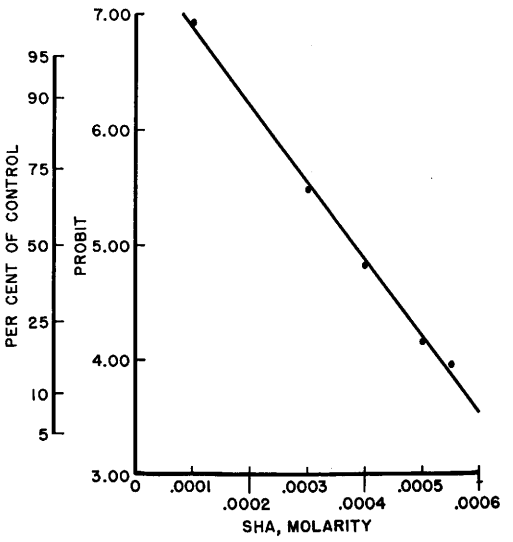

FIG. 1. Dose-response relationship of DNA synthesis by Ehrlich ascites tumor cells to salicylhydroxamic acid. The line was calculated by the method of least squares.

value varied only slightly in a number of experiments. Linearity of response was achieved by plotting probit of % of control against the concentration rather than against the logarithm of the concentration. The regression coefficient of the line was consistently large. The value calculated from the data in Fig. 1 was —6719, or a decrease in 1 probit unit for each increment of concentration of approximately 1.5×10^{-4}M.

The selectivity of inhibition of DNA synthesis by SHA is depicted in Fig. 2 and 3. In these experiments the rates of synthesis of DNA, RNA, and protein were measured simultaneously with and without SHA at 10^{-3} M. When cells were not preincubated with SHA prior to addition of the isotopic precursor but received the two simultaneously (Fig. 2), DNA synthesis was markedly depressed, while RNA synthesis was virtually unaffected and protein synthesis was stimulated to a slight extent. When the cells were preincubated with SHA for 2 hours prior to addition of isotopes, the pattern of response over the subsequent 1 hour sampling period was virtually the same, except RNA synthesis was nominally depressed and the rate of protein synthesis was indistinguishable from the control value. Preincubation of the cells

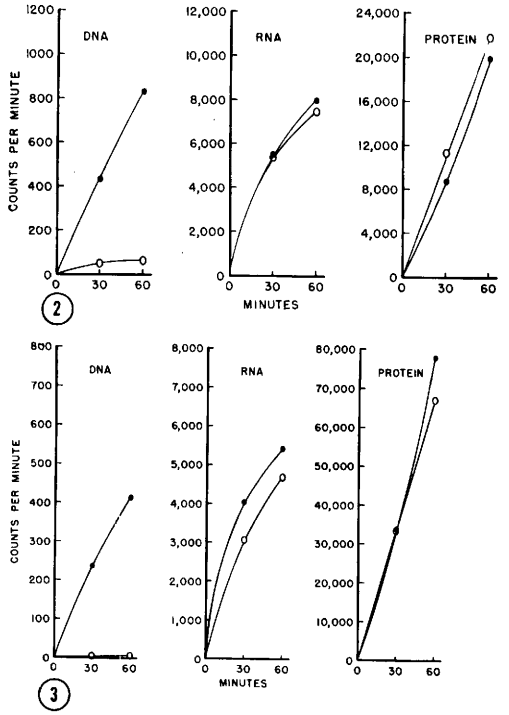

FIG. 2. Effect of salicylhydroxamic acid (SHA) on DNA, RNA, and protein synthesis by Ehrlich ascites tumor cells. SHA and isotopic precursors were added to the cells simultaneously. Closed circles, controls; open circles, with 10^{-3} M SHA.

FIG. 3. Conditions the same as in Fig. 2, except the cells were preincubated with SHA for 2 hr before addition of isotopes. Closed circles, controls; open circles, with 10^{-3} M SHA.

with SHA for 1 hour prior to addition of isotopes yielded curves intermediate between those in Fig. 2 and 3.

Reversibility of inhibition is demonstrated in Fig. 4. Following preincubation of the cells for 30 minutes with and without SHA, those preincubated in its absence were washed 3 times with fresh medium. Those preincubated with SHA were divided into 2 equal aliquots; one was washed 3 times with fresh medium without SHA, while the other was washed with fresh medium containing SHA at the same concentration as was present during the 30-minute preincubation period. Thymidine-^{3}H was then added and the rate of DNA synthesis was measured as previously. The inhibition conferred by 5×10^{-4} M SHA was completely abolished by removal of the inhibitor, while the effect induced by a concentration of 10^{-3} M was markedly reduced.

To determine if other closely related compounds were inhibitory to nucleoside assimilation, cells were preincubated for 1 hour with 2 concentrations of salicylamide, salicylic acid, or salicylhydrazide. Synthesis of DNA following exposure to each was determined as before and the results are shown in Table I. Salicylamide and salicylic acid were only slightly active in this respect; salicylhydrazide was somewhat more active, but yielded only 34% inhibition at 10^{-3} M.

Discussion. The foregoing data demonstrate inhibition by salicylhydroxamic acid (SHA) of DNA synthesis in Ehrlich ascites tumor cells *in vitro*. The characteristic features of this action of SHA are (a) rapidity of onset, (b) selectivity for DNA synthesis with little or no depression of RNA or protein synthesis at the concentrations tested, and (c) reversibility upon removal of the inhibitor. It is noteworthy that each of

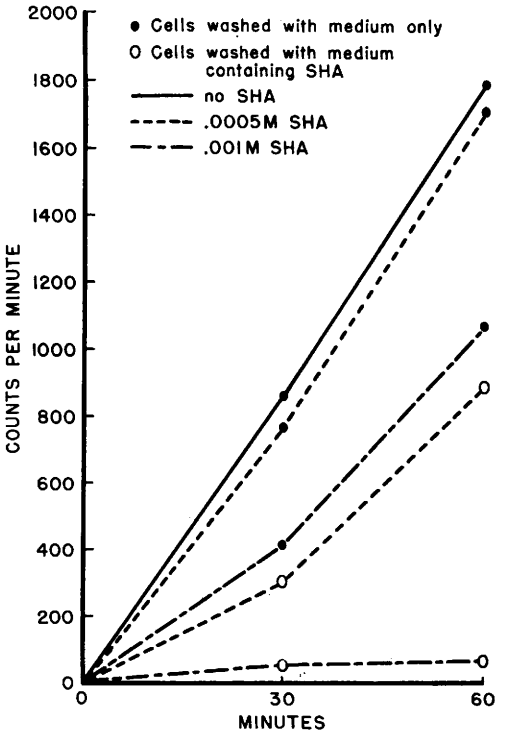

FIG. 4. Reversal of inhibitory action of salicylhydroxamic acid (SHA) after removal of the inhibitor by washing the cells. Experimental conditions described in text.

TABLE I. Effects of Salicylhydroxamic Acid and Certain Related Compounds on Rate of DNA Synthesis by Ehrlich Ascites Tumor Cells.

Compound and concentration	% inhibition
Salicylhydroxamic acid, 5×10^{-4} M	70
" " , 10^{-3} M	92
Salicylamide, 5×10^{-4} M	4
" , 10^{-3} M	10
Salicylic acid, 5×10^{-4} M	10
" " , 10^{-3} M	18
Salicylhydrazide, 5×10^{-4} M	18
" , 10^{-3} M	34

these characteristics is shared by hydroxyurea (3,8,9), the hydroxamic acid derivative of carbamic acid which is currently receiving clinical trials in various neoplastic states (see 10 for extensive bibliography).

The Cumulative Index of Materials(11) recently compiled by the Cancer Chemotherapy National Service Center lists 37 entries of compounds with the empirical formula $NO_3C_7H_7$ which have been screened for potential antitumor properties. Two of these entries were subsequently found to pertain to SHA, and the compound had been assigned the National Service Center number NSC 5088. In the first test for activity(12) SHA was screened against sarcoma 180, mammary adenocarcinoma 755, and leukemia L-1210. The compound was considered as yielding negative results since the survival ratio of the treated animals as compared with controls (T/C) was only 1.6 in the case of leukemia L-1210; the minimum acceptance level in that system was a T/C of 1.7. Results with sarcoma and adenocarcinoma were also considered to be negative. The dosage schedules used ranged from 35 mg/kg/day to 500 mg/kg/day. A subsequent test some time later using sarcoma 180 and SHA at 500 mg/kg/day for 13 days likewise yielded negative results(13). In spite of lack of demonstrated effectiveness of SHA in the experimental tumor systems, it appears that dosage levels as high as 500 mg/kg/day are relatively non-toxic.

In each of the tumor test systems SHA was administered as a single daily injection, except in the earlier of the two reports(12) in which it was given as 2 injections per day when screened against sarcoma. Of interest in this connection is the report by Lowenthal (14) concerning the metabolism of SHA. He found that rat liver extracts rapidly reduce SHA to salicylamide; no evidence of hydrolysis of the former to salicylic acid and hydroxylamine could be found. Salicylamide was also recovered as a conjugate from the urine of intact rats receiving SHA. Although a precise study of the rate of the reduction was not done, chromatography revealed a significant amount of SHA reduced after only 30 minutes incubation with liver extracts, and the amount appeared to increase during the 4-hour incubation period. In view of the relatively rapid rate of biotransformation of a selective inhibitor of DNA synthesis (SHA) to a well-known analgesic agent with low toxicity (salicylamide)(15), further testing of SHA in the experimental leukemia L-1210 system appears essential, employing multiple daily injections rather than a single injection.

Any possible relationship between the observed slight inhibition of DNA synthesis by salicylic acid and the teratogenic action of certain salicylates(16-18) remains to be clarified.

Summary. Salicylhydroxamic acid (SHA) was shown to be a selective inhibitor of DNA synthesis by Ehrlich ascites tumor cells *in vitro*. Inhibition was evident virtually immediately after addition of the compound to the cells; no preincubation was necessary to evoke the effect. Cells which were preincubated with SHA for up to 2 hours showed only a slight depression of RNA synthesis, while the rate of protein synthesis was unaltered. Reversal of the inhibition upon removal of SHA was complete at lower concentrations, and partial at higher concentrations.

The skilled technical manipulations of Alayne B. Smith facilitated many phases of this work.

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Effectiveness of THAM in Preventing Cellular Damage Resulting From Oxygen Lack.* (31370)

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Intracellular acidity increases during oxygen lack in a variety of tissues. It has been suggested that this decrease in pH may destroy enzymes and disrupt metabolic function within the cell. The damaging effects of the increased acidity might be lessened if acids were neutralized by a buffer capable of accepting hydrogen ions within the cell. The organic buffer tris-hydroxy methylamino methane (Tris or THAM), is capable of acting as an intracellular buffer(1). THAM buffer had been shown to be more effective in correcting acid-base disturbances, *e.g.*, apneic oxygenation and hypovolemic shock, than carbonate buffers(2,3). Thus the following study was undertaken to determine whether the organic buffer THAM could attenuate the cellular damage associated with oxygen lack. The criteria used to establish the severity of cell damage included: (a) reduction in the activities of selected mitochondrial and microsomal enzymes, (b) labilization of lysosomal enzymes (measured as increased per cent free activity) and (c) degradation of protein (measured by an increased concentration of soluble nitrogen).

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Methods. Male rats of the Sprague-Dawley strain weighing 150-300 g were fed *ad libitum* on Purina Laboratory Chow and water. At the time of death, the animals were decapitated with a guillotine. The left lateral and median lobes of the liver were used for all enzymatic and chemical determinations. The animals were divided into the following groups.

Group A—normal controls.

Group B—liver autolysis. Anoxia was produced by incubation of isolated lobes in a nitrogen atmosphere, at 37°C, moistened with Krebs-Ringer phosphate pH 7.2 for 1 or 12 hours.

Group C—administration of THAM buffer IP followed by liver autolysis. Ten ml of 0.33 M THAM (tris-hydroxy methlyamino methane), pH 7.2, was injected IP. After 18 hours the rat was sacrificed and the liver was allowed to autolyze as in Group B.

Group D—control liver slices for *in vitro* experiments. Liver slices were prepared with a Stadie-Riggs hand microtome. The slices