likely that the hormones only inhibit or mask the peripheral manifestations of rheumatoid arthritis, rheumatic fever, lupus erythematosus and psoriasis and do not affect the underlying causative disease mechanism, since a clinical relapse usually occurs promptly after cessation of hormone treatment.(2,4) In acute gouty arthritis, on the contrary, ACTH apparently completely aborts the acute attack.(1)

There are none of the usual signs or symptoms of adrenal insufficiency in the diseases benefitted by ACTH or Compound E and it is unlikely that the hormones act as substitution therapy for a deficiency. The expected change in the ketosteroids, eosinophils, lymphocytes, and total white count is evidence for adequate adrenal cortical function.(6) A "pharmacologic" extension of the usual physiologic properties of the hormones may be responsible for their action. In this connection, it is interesting to note the similarities between the effects produced by the salicylates and the adrenal hormone. Both groups of compounds lower fever and sedimentation rate, inhibit hyaluronidase,(7,8) cause hyperglycemia,(6,8) increase uric acid excretion(9,10) and are local anesthetics.(8,11) In addition, the salicylates and the adrenal hormones are both effective in rheumatoid arthritis and lupus erythematosus.(8)

Summary. Two patients with classical disseminated lupus erythematosus were given three separate courses of injections of adrenocorticotropic hormone (ACTH). In each case, there was temporary, striking clinical improvement and fading of the skin lesions, accompanied by the expected signs of increased adrenal cortical activity. These results suggest that continued administration of ACTH or Compound E may be of benefit in lupus erythematosus.

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A Test of Triazolopyrimidines on Mouse Sarcoma 180.* (17500)

C. CHESTER STOCK, LIEBE F. CAVALIERI, GEORGE H. HITCHINGS AND SONJA M. BUCKLEY

From the Sloan-Kettering Institute for Cancer Research, New York City, and the Wellcome Research Laboratories, Tuckahoe, N. Y.

For nearly two years the major portion of a cancer chemotherapy program(1) has been devoted to a study of compounds that might serve as anti-metabolites to nucleic acid components. The studies were stimulated by the development of knowledge concerning the effects of anti-metabolites in bacteria and

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animals(2-5) and the increasing information regarding details of nucleic acid metabolism.(6-8) Approximately 25 purines, 200 pyrimidines, 50 pteridines, 40 folic acid analogs and numerous miscellaneous heterocyclic compounds have been subjected to test. Among the compounds found to be most active in this program are those with anti-folic acid activity(9) and 2,6-diaminopurine(10) for the prolongation of survival of leukemic mice and some folic acid analogs for the inhibition of Sarcoma 180 and a few other tumors.(11-13) Compounds showing less inhibition of Sarcoma 180 in repeated tests have been found in each of the categories mentioned above. The reproducible, weak effects have been of interest in providing leads for study of related compounds.

A number of triazolo[*d*]pyrimidines (8-azapurines)(14) were among those which failed to show a satisfactory inhibitory effect in tolerated amounts against Sarcoma 180. The recent report(15) of the inhibitory action of 8-azaguanine (5-amino-7-hydroxy-l-v-triazolo [*d*]pyrimidine), also referred to as "guanazolo," on mouse mammary adenocarcinoma EO 771, on spontaneous mammary cancer in C3H mice, and on lymphoid leukemia in strain A mice redirected attention to our findings. The action of the guanine analog was interpreted by Kidder as indicating a similarity in guanine metabolism between *Tetrahymena gelii* and neoplastic tissues and, a "uniformity of various cancer tissues with respect to guanine metabolism".(15) An extension of our experiments with 8-azaguanine, 8-aza-adenine, 8-aza-isoguanine, 8-aza-2, 6-diaminopurine and 8-azaxanthine against Sarcoma 180 under conditions favoring demonstration of inhibitory action confirmed the ineffectiveness of these purine analogs at safe dosages. Inhibition was observed with each compound only when it was given in amounts sufficient to kill a large percent of the mice.

Materials and methods. Samples of the triazolopyrimidines were prepared independently by two of the authors, (L.F.C.)(16) and (G.H.H.). Dr. M. L. Crossley of the Calco laboratories kindly supplied two additional samples of 8-azaguanine. The technic used in the Sarcoma 180 test has been described.(11) In brief, small tumor implants (1-2 mm in any dimension) were made subcutaneously by trocar into the axillary region of CFW or RF mice, 18-22 g in weight. Twenty-four hours later the compounds suspended in gum acacia were injected intraperitoneally twice daily for one week. The dosages used were based initially on maximum tolerated doses determined by Dr. F. S. Philips, Pharmacology Section. Higher toxic doses were subsequently used when inhibition was not found at the safe levels. At the end of the injection period, the tumors were measured with calipers in two diameters. The degree of inhibition of tumor growth was graded as follows:

Marked inhibition (+), no growth of tumors in treated animals to a growth with an average diameter ¼ that of the untreated controls;

Slight inhibition (±), growth of tumors in treated animals from ¼ to ¾ the average diameter of the control tumors;

No effect (−), growth af tumors greater than ¾ the average diameter of the controls.

Discussion of results. The data are summarized in the table for only the highest levels tested with each compound. At lower toxic levels the compounds inhibited the tumors slightly and at non-toxic levels there was no effect. None of 8 samples of 8-azaguanine inhibited the development of Sarcoma 180, when tested at non-toxic levels. These levels,

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TABLE I.
Results with Triazolopyrimidines Tested Against Sarcoma 180.

SK No.	Name	Dose, mg/K/day	No. of mice*	No. of deaths	Tumor† inhibition
1054	5,7-diamino-l-v-triazolo (d)pyrimidine	250	20	9	— to ±
1059	5-hydroxy-7-amino-l-v-triazolo (d)pyrimidine	100	15	8	±
1149	5,7-dihydroxy-l-v-triazolo (d)pyrimidine	1500	15	8	— to ±
1150	5-amino-7-hydroxy-l-v-triazolo (d)pyrimidine	250	35	7	— to ±
1315	7-amino-l-v-triazolo (d)pyrimidine	125	35	23	— to ±

* A total of 410 treated animals were used in test groups of 5 mice each. Data for only the highest doses are tabulated. The remainder of the mice were tested at lower levels of the compounds as follows: SK 1054, 128-200 mg/K/day, 60 mice; SK 1059, 16-70 mg, 65 mice; SK 1149, 500-1000 mg, 55 mice; SK 1150, 64-200 mg, 85 mice; SK 1315, 63-100 mg, 25 mice. Two or more preparations of each compound were used.

† The grading of tumor inhibition is described in the section on Materials and Methods.

64-150 mg/K/day, were higher and initiated earlier (24 hours after tumor implantation) than in the experiments with adenocarcinoma EO 771 reported elsewhere. The preparations of 8-azaguanine varied in purity from one containing 40% of the guanine analog and 60% of the xanthine analog to several containing only one component as judged by a paper chromatogram.† The tumor inhibitory effects with the several samples of 8-azaguanine tested at 200 and 250 mg/K/day are considered insignificant because they were obtained at lethal levels. The analogs of isoguanine, 2,6-diaminopurine, adenine and xanthine also showed inconsistent inhibitory effects but only at lethal levels.

The failure of 8-azaguanine to inhibit selectively the growth of Sarcoma 180 is inconsistent with the concept that tumor cells in general possess a guanine metabolism comparable to that demonstrated for tetrahymena.(15) Sarcoma 180 would appear to be the first exception to the stated concept of "uniformity of various cancer tissues with respect to guanine metabolism".(15) The difficulties in generalizations concerning purine metabolism are further demonstrated by the observation(17) that with C 57 black mice bearing adenocarcinoma EO 771 there is an incorporation of guanine into nucleic acids of both normal and tumor tissue whereas in normal rats no significant incorporation has been observed.(6,7)

Regardless of the negative results obtained with 8-azaguanine against Sarcoma 180, it has been considered essential to test the compound in a spectrum of tumors. The results of these tests to be reported shortly(18) will reveal whether Sarcoma 180 possesses a metabolism unique among tumors with respect to guanine or merely represents a difference in response of Sarcoma 180 and mammary adenocarcinoma EO 771 to certain compounds such as found previously with folic acid analogs.(13)

Summary. 8-Azaguanine and 4 other triazolopyrimidines at tolerated doses were without inhibitory effect on Sarcoma 180.

† We are indebted to Dr. Aaron Bendich of the Protein Chemistry Division of Sloan-Kettering Institute for this analysis.

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