as the compounds mentioned here as showing therapeutic activity, caused a definite fall in the leukemic leukocytes in the peripheral blood. Clinical trials with SK 1133 (triethylene melamine) have shown it to possess chemotherapeutic activity similar to that of methyl-bis(β-chloroethyl)amine against Hodgkin's disease, lymphosarcoma, and the chronic leukemias(13). It would seem valid, therefore, to attribute the activity as chemotherapeutic agents, of the particular series of compounds discussed in this report, to the nitrogen mustard-like ethylenimine ring rather than to the triazine or melamine structure.

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Summary. 1. 42 compounds, of which 39 could be classed as triazines and 3 as ethylenimine ring compounds were screened for chemotherapeutic activity against Ak4 mouse leukemia. 2. 2,4,6-triethylenimino-*s*-triazine, 2, 4 - diethylenimino - 6 - amino - *s* - triazine, and hexamethylene diethylenurea significantly prolonged the survival time of mice injected with transplanted leukemia Ak4. 3. It would appear that the chemotherapeutic activity of the above compounds can be attributed to the ethylenimine structure. 4. Four other compounds containing either a triazine or an ethylenimine moiety showed a slight chemotherapeutic effect.

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Inactivation of Estradiol by the Hepatic Tissues of Mice.* (18023)

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Because of the role that estrogens play in experimental carcinogenesis and in many clinical conditions, considerable significance is attached to any factor which would increase or decrease the titer of circulating estrogens. The liver destroys estrogen in a majority of laboratory animals(1-4). Marked damage to the liver in these animals caused a rise in titer of the circulating estrogen(5,6). Factors which will decrease the liver's ability to inactivate estrogen include carbon tetrachloride toxicity(5), virus hepatitis(7), partial hepatectomy(6), and nutritional deficiency(8,9). An important aspect of this problem is: what is the effect of large quantities of estrogen in the blood upon the liver's ability to inactivate estrogen?

The effect of a prolonged castrate condition and of prolonged hyperestrogenemia upon the ability of the mouse's liver to inactivate estrogen was studied.

Methods. Animals used were first generation hybrid mice, designated CC_1, CC_2, AC_1, AC_2, AC_3, and AC_4. A few inbred mice of the C_{57}, NHO and CHI strains were used. The mice were divided into 3 groups, one group was untreated, mice of the second group were castrated, and mice of the third group were castrated and then 2 mg pellets of estrone were implanted subcutaneously. Six weeks, 8

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TABLE I.
Hepatic Inactivation of Estradiol in Mice.

Condition of animals	Substrate	No. of animals	Mean age (mo.)	Mean QO_2	Amt of estradiol inactivated, m.u./mg dry wt
Normals	Without estradiol	7	2½	5.1 ± 1.20	—
,,	With ,,	13	2½	5.1 ± 0.83	8.5 ± 1.05
Castrated 6 wks	,, ,,	5	3½	5.2 ± 0.73	5.6 ± 0.88
Castrated and Imp.* 6 wks	,, ,,	5	3½	5.4 ± 0.71	5.5 ± 0.95
Castrated 8 mo.	,, ,,	8	12	4.7 ± 0.83	6.3 ± 1.05
Cast. and Imp. 8 mo.	,, ,,	8	11½	5.4 ± 0.59	6.6 ± 0.84
Castrated 18 mo.	,, ,,	4	21½	5.3 ± 0.54	6.5 ± 1.14

* Imp.—refers to implantation of pellets of estrone.

months, and 18 months following castration or castration and the implantation of pellets the mice were killed, their livers removed, and the activity of their hepatic tissues in inactivating estradiol was tested. Thin slices of liver were cut and placed on a solution containing a known amount of estradiol in a Warburg flask for a period of one hour(10). The flasks were then placed in boiling water for 10 minutes to destroy enzymatic activity. The amount of estradiol remaining at the end of one hour was then estimated by bio-assay (11). The estradiol was dissolved in a Krebs-Ringer-phosphate buffer solution(12). The liver was sliced between 0.4 and 0.6 mm in thickness, a size which is optimal for hepatic tissue used in manometric studies(13). The Warburg flasks were shaken at a rate of one hundred times per minute and maintained at a temperature of 37.5° ± 0.02°C.

All assays were carried out by the vaginal smear method. A total of 12 or more mice were used for each assay. The entire contents of the Warburg flasks, including the liver slices, were homogenized and injected in fine suspension.

Results. The results of these experiments may be appraised by referring to the accompanying table and graph. In order to determine whether hepatic tissue has any estrogenic activity, seven experiments were performed in which homogenized hepatic tissue from mice was bio-assayed. Amounts of mouse's liver ranging from 12.0 mg to 39.0 mg showed no estrogenic activity in these bio-assays.

Mice which were castrated and implanted with pellets of estrone lost an average of 3 g in weight. Mice which were castrated but not implanted with estrogen gained an average of 4 g before they were sacrificed.

Discussion and conclusion. The incubation of mouse's liver with estradiol did not raise the Qo_2 of the hepatic tissue. Moreover a decrease in the ability of the hepatic tissue from mice to inactivate estradiol *in vitro* was not accompanied by a change in the Qo_2 of the liver slices. Although the inactivation of estradiol is generally thought to be an oxidation reaction(14) the oxygen uptake of the inactivation reaction was too small to be detected by the Warburg technic.

One milligram by dry weight of mouse's liver inactivated 8.5 mouse units of estradiol in one hour under the experimental conditions used. This figure represents the maximum amount of estradiol that one milligram of mouse's liver inactivated. If less than this concentration of estradiol was present, all the estradiol present was inactivated. If more than 8.5 m. u. of estradiol per milligram of hepatic tissue was present, increasing amounts of estradiol remained unchanged in solution as the concentration of estradiol was increased.

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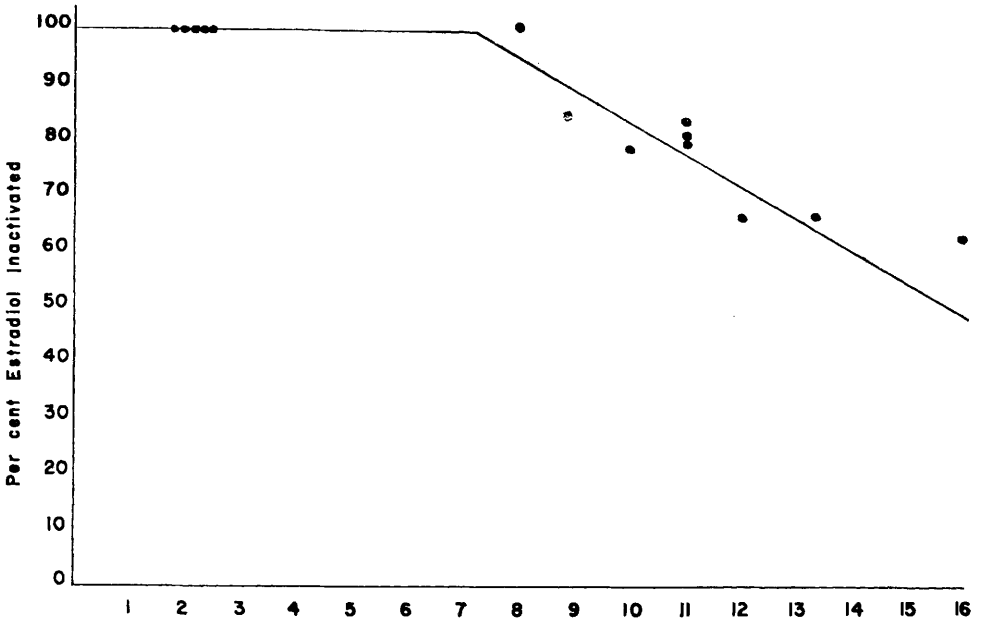

FIG. 1.

The maximum rate of inactivation of estradiol by hepatic tissue in these experiments was thus unaffected by the concentration of estradiol present (see graph).

The capacity of liver slices from castrated mice to inactivate estradiol was significantly below that of intact, untreated mice. This fall in inactivating capacity was observed within 6 weeks after operation and was maintained for 18 months. Slices of liver from mice that were castrated and received pellets of estrone showed a decrease in estradiol inactivating capacity similar to that found in castrated mice. It would seem from this that the fall in inactivating capacity of the mouse's hepatic tissue was the result of castration alone and that the addition of pellets of estrone did not alter the change. On the other hand, it is possible that the animals implanted with pellets of estrone achieved the changes in hepatic inactivation through a somewhat different mechanism.

Weight loss had little effect on the loss of estradiol inactivating ability of the hepatic tissue. The same changes in hepatic inactivation of estradiol were observed in the livers of mice which were castrated and gained weight and in the livers of the mice which were castrated and implanted with estrone and lost weight.

Summary. Hepatic tissue of normal mice inactivated 8.5 m.u. of estradiol per mg dry weight per hour. Hepatic tissue from castrated mice or from estrone-treated mice was slightly less effective.

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