reactions to hormonal stimuli observed in the two species. Thus, thiouracil has been shown to decrease body growth(3) and mammary growth(4,5) in mice, but increase body growth(6) and mammary growth(5,7) in rats. Thyroid administration has been demonstrated to increase body growth(8) and mammary growth(9) in mice, but decrease body growth (8) in rats. It is not definitely known how thyroidal substances exert their modifying influences on the reaction of the gonads of rats and mice to PMS. A recent report by Warner and Meyer(10) indicates that in rats the administration of thyroxine probably acts directly on the ovaries to inhibit their reaction to endogenous gonadotrophins.

Summary. The effects of experimentally induced hypo- and hyperthyroidism on the ovarian response to pregnant mares' serum were determined in immature rats and mice by administering thiouracil for 4 to 20 days and thyroxine or thyroprotein for 10 days. A constant dose of PMS was injected into each animal during the last 4 days of each experiment and the increase in ovarian weight was measured. It was found in rats that all doses of thyroprotein or thyroxine significantly decreased the action of the gonadotrophin on the ovaries, while in mice thyroprotein significantly increased the ovarian response to PMS. When thiouracil was fed to rats for 4, 7, 10 and 15 day periods, there were significant increases in ovarian response to PMS, but when fed for 20 days thiouracil reduced the ovarian response to PMS. These data on young female rats and mice corroborate the results previously obtained in young male rats and mice. It is suggested that insofar as the ovarian response to PMS is concerned, young rats of both sexes secrete more and young mice less than an optimal amount of thyroid hormone.

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Effect of 5-Amino-7-Hydroxy-1H-v-Triazolo (d) Pyrimidine on Growth and Development of the Chick Embryo.* (18130)

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In 1949, Kidder and his associates(1) reported that the growth of the protozoan, *Tetrahymena gelii,* and certain mammalian tumors was inhibited by the guanine analogue, 5-amino-7-hydroxy-1H-v-triazolo (d) pyrimidine (guanazolo).† Other investigators have studied the effect of this agent on a wider range of neoplasms and considerable variation has been observed in the response of different tumors to it(2-4).

The purpose of this communication is to

* This study was supported by a grant from The Charles F. Spang Foundation, Pittsburgh, Pa.

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† The alternate designation, "8-azaguanine", has been suggested for this compound(3).

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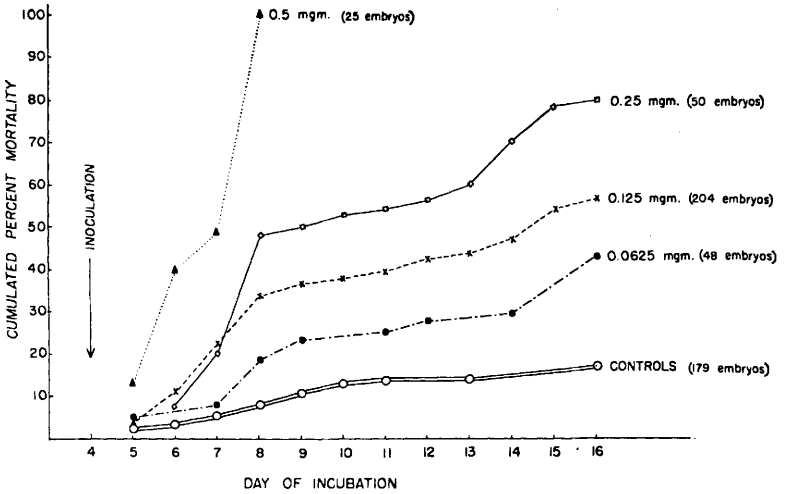

FIG. 1.

Cumulated percent mortality of chick embryos at intervals following yolk sac inoculation with different doses of guanazolo.

present observations on the toxicity of guanazolo for the chick embryo. This investigation was undertaken after it was noted in preliminary studies(5) that even small quantities of this compound affected embryos, in contrast to the reported absence of effect upon the host in studies in mice(1).

Methods. Fertile white leghorn eggs were incubated at 38.5°C and a relative humidity of 85%. Test solutions in 0.1 cc or 0.2 cc volumes were injected into the yolk sac by introducing a 1¼ inch, 23 gauge needle through a hole in the blunt end of the egg. Guanazolo‡ solutions were prepared by dissolving 100 mg of the chemical in 2.0 cc of 1N NaOH and then diluting in distilled water to the desired concentration. Eggs were candled daily; dead embryos were removed, weighed, and examined. Experiments were usually terminated when the embryos were 16 days of age. All surviving embryos were sacrificed, weighed, and examined.

Results. In preliminary observations, made in the course of studies on effect upon virus

TABLE I. Weight Averages and Incidence of Abnormalities in 16-day-old Chick Embryos Inoculated on 4th Day of Incubation with Guanazolo or Various Doses of Guanine HCl plus Guanazolo.

Group	Inoculum (in .1 or .2 cc)	No. embryos	Avg wt, g	Range, g	Embryos showing abnormalities	
					No.	%
1	Controls (H_2O)	76	12.8	8.4-16.1	2	2.6
2	Guanazolo (.125 mg)	66	9.6	4.0-14.4	20	30.3
3	Guanazolo (.125 mg) + *Guanine HCl* (4.0-8.0 mg)	28	11.3	8.9-14.0	1	3.5
4	Guanazolo (.125 mg) + *Guanine HCl* (2 mg)	41	11.5	6.0-15.7	4	9.5
5	Guanazolo (.125 mg) + *Guanine HCl* (.5-1 mg)	47	12.0	6.0-15.6	6	13.7
6	*Guanine HCl* (8 mg)	26	12.2	8.5-15.2	0	0

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‡ The guanazolo used was furnished by the American Cyanamid Co., Lederle Laboratories Division, through the courtesy of Dr. J. M. Ruegsegger.

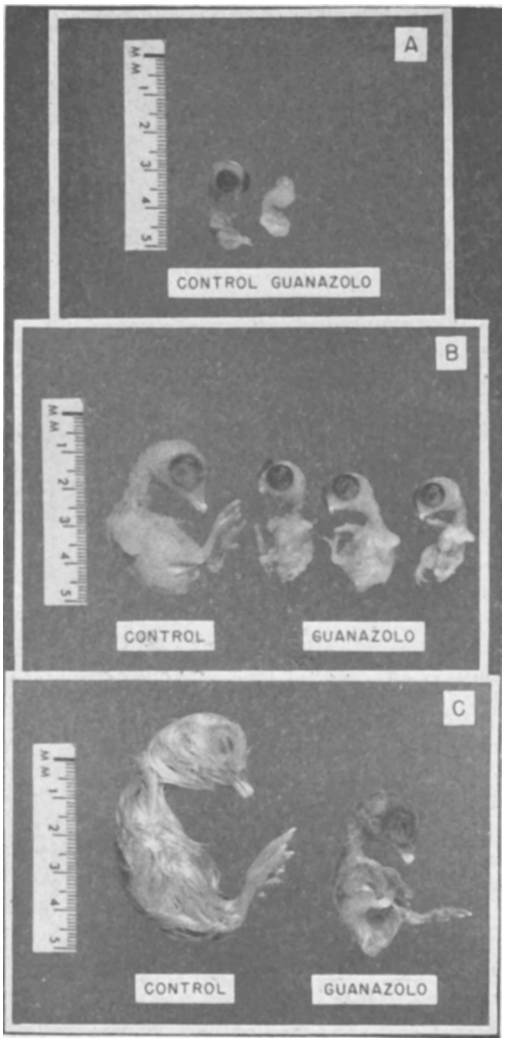

FIG. 2.

Appearance of chick embryos treated on 4th day of incubation with 0.125 mg of guanazolo and sacrificed at various intervals. Comparison with control embryos of similar ages. A—8th day of incubation; B—14th day of incubation; C—16th day of incubation.

multiplication, 10-day-old embryonated eggs were inoculated with guanazolo introduced into the allantoic sac or the yolk sac. Only in the latter instance did slight inhibition of growth occur. Deaths were noted at higher dosage levels (0.5 mg/egg). Corresponding dosages given allantoically were less toxic. Younger embryos were found to be more sensitive to the action of guanazolo, and in the majority of experiments here reported inoculations were made on the 4th day of development. Embryos younger than 4 days of age did not survive toxic doses long enough for developmental abnormalities to occur.

The increased mortality resulting from injections of 0.1 cc of solutions containing different amounts of guanazolo given into the yolk sac of 4-day-old embryos is illustrated in Fig. 1. As can be seen from this graph, the mortality by the 16th day of incubation produced by 0.5, 0.25, 0.125 and 0.0625 mg doses of the drug was 100%, 80%, 56% and 43% respectively. The LD_{50} was approximately 0.125 mg/egg. The control inoculum consisted of sterile distilled water. The small amount of alkali present in the different guanazolo concentrations employed was found to have no effect on growth or development when compared with untreated controls. This is not surprising since it has been found that normal yolk, early in development, is acid in reaction. In addition, the strong buffering action of the yolk and dilution of the inoculum would preclude significant changes in pH.

Surviving embryos at the 16th day of incubation were much smaller than the controls of corresponding age (Table I, Groups 1 and 2), and they exhibited a higher rate of developmental abnormalities. The abnormalities found were restriction both of vascularization and development of the chorioallantoic membrane, abnormal beaks, edema of the skin and peritoneal cavity, exteriorization of viscera, deformed extremities, inhibition of feather formation, and hemorrhage. Photographs illustrating some of these changes in treated embryos at different stages of development are shown in Fig. 2.

In order to determine whether the effects of guanazolo in chick embryos were the result of a generalized toxic action or, more specifically, an interference with normal guanine metabolism, the influence of guanine HCl on guanazolo inhibition was studied. Guanine HCl was dissolved in 1N NaOH (80 mg/cc) and desired concentrations prepared by diluting with sterile distilled water. Alkali alone, in quantities corresponding to that inoculated with guanine, had no effect on growth or development when compared

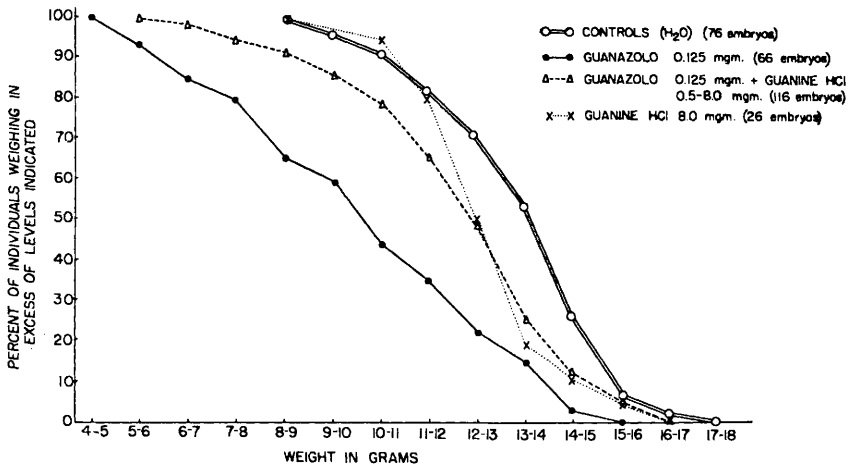

FIG. 3.

Comparison of weight distribution of 16-day-old sacrificed embryos treated on the 4th day of incubation with guanazolo alone, with guanine HCl alone, or with guanazolo plus guanine HCl. Weights plotted cumulatively as percent of individuals weighing in excess of indicated levels.

with untreated control embryos. Doses of guanine HCl ranging from 0.5 to 8 mg were injected into the yolk sacs of 4-day-old embryos together with 0.125 mg of guanazolo. As controls, groups of embryos were inoculated either with guanazolo or guanine HCl. Evidence of interference with guanazolo inhibition was obtained with all of the doses of guanine HCl employed. As can be seen in Table I, embryos that received guanine HCl plus guanazolo on the 4th day of incubation and were sacrificed on the 16th day of incubation (Groups 3, 4 and 5) were comparable in weight to the controls (Group 1). The distribution of weights of embryos inoculated with guanazolo alone (Group 2) was significantly lower.

In Fig. 3 is shown the weight distributions of individual embryos in the different groups. The points on the curve represent the percent of individuals in each group weighing in excess of the levels indicated. All embryos that had been inoculated with guanazolo plus guanine HCl were grouped together, regardless of the dose of guanine HCl. The proximity to that of the control of the curve for embryos given guanine plus guanazolo, and the relationship of both to the curve for embryos treated with guanazolo alone, suggests that guanine HCl, when injected *together with* guanazolo, interfered with the growth inhibiting effect of the latter. In addition, developmental abnormalities were less frequent when guanine HCl was injected along with guanazolo (Table I).

Discussion. From the results presented it is clear that 5-amino-7-hydroxy-1H-v-triazolo (d) pyrimidine (guanazolo) even in very low concentration, has a profound effect on the growth and development of the chick embryo. The inhibition of the guanazolo effect by guanine HCl suggests that the chick embryo requires guanine but may not be able to synthesize this purine. These findings suggest a similarity in the nucleic acid metabolism of the chick embryo, *Tetrahymena*, and certain mammalian tumors. There are many indications of similarities in various metabolic systems in embryonic and tumor tissues(6) and the results reported here may indicate still another relationship. The specificity of the guanazolo effect is being tested further with adenine and embryos showing the effects of guanazolo treatment are being studied histologically.

Summary. The guanine analogue, 5-amino-7-hydroxy-1H-v-triazolo (d) pyrimidine (guanazolo), which has been reported to be relatively non-toxic for adult mice, was found to be toxic for the developing chick embryo.

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This was manifested by a high mortality rate, inhibition of growth, and the occurrence of developmental abnormalities. The toxic effects of guanazolo were inhibited by the simultaneous injection of guanine HCl.

The technical assistance provided by Miss Doris M. Larsen in the beginning of this study is gratefully acknowledged.

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Administration of the Hyperglycemic-Glycogenolytic Factor of the Pancreas to Non-Anesthetized and Anesthetized Subjects. (18131)

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That a hyperglycemic property may be present in pancreatic extracts and in commercial preparations of insulin has been recognized for some time. The recent preparation of a relatively purified hyperglycemic-glycogenolytic factor (HGF) from the pancreas and from gastric mucosa by Sutherland and DeDuve(1) and the demonstration of consistently reproducible *in vitro* and *in vivo* effects, have led to speculations concerning the possible role of such a factor in the regulation of carbohydrate metabolism in man, and particularly in the genesis of human diabetes. Nevertheless, Weisberg, Friedman and Levine(2) after summarizing the pertinent literature and from their own observations concluded that "as of the present, there has been no clear cut demonstration to prove that HGF is a mormone and/or that it is secreted by the alpha cell or the argentophil cells."

In view of the frequent presence of HGF in commercial preparations of insulin utilized in the treatment of diabetes mellitus in man, it was deemed advisable to assay the effects of the administration of a preparation rich in HGF to man. Since anesthesia has been shown to enhance the effect of the administration of HGF to rabbits(2), the influence of this factor in men, with and without anesthesia was determined. It was deemed pertinent also to compare the response in man with that of other species.

Method. A preparation of insulin which was relatively rich in hyperglycemic factor was prepared by one of us (E.D.C.). It is estimated that this preparation contained that amount of HGF as would be present in an equal weight of commercial insulin. Large numbers of assays with cats and other animals revealed that as little as 0.05 mg per kg body weight produced a marked rise in blood sugar. The effectiveness of this preparation was not impaired by filtration through an ultra-fine fritted Pyrex disc bacterial filter. Before each experiment, a fresh solution containing 0.5 mg HGF per ml acid water (pH 2.5) was prepared and sterilized by fritted-glass filtration. After a preliminary period of 20 to 30 minutes during which blood samples were drawn, 0.1 ml of the HGF solution per kg body weight was injected intravenously. Subsequent specimens of venous blood were withdrawn at five-minute intervals for thirty minutes. In some instances simultaneous arterial samples were drawn. The majority of the subjects were ambulatory patients on the psychiatric wards and varied from 17 to 53 years in age. In addition, 2 patients with diabetes of mild severity were tested. In 8 patients, the experiment was performed both without anesthesia and with anesthesia. Altogether, 21 experiments were

* Post-doctorate Research Fellow of the National Institutes of Health.

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