This material removes nonspecific properties, does not markedly reduce or increase antibody titers with sera giving specific reactions with virus and rickettsial antigens, and does not introduce nonspecific properties in nonreacting specimens.

1. Sachs, H., *Berlin Klin. Wochschr.*, 1921, v58, 1075.

2. Maltaner, F., and Maltaner, E., In N. Y. State Department of Health, Division of Laboratories and Research, *Ann. Rep.*, 1943, p19.

3. ———, *ibid.*, 1945, p14.

4. Davis, B. D., Kabat, E. A., Harris, A., and Moore, D. H., *J. Immunol.*, 1944, v49, 223.

5. Davis, B. D., Moore, D. H., Kabat, E. A., and Harris, A., *ibid.*, 1945, v50, 1.

6. Taran, A., *J. Lab. Clin. Med.*, 1946, v31, 1037.

7. Maaløe, O., *On the Relation Between Alexin and Opsonin. Dissertation,* Munksgaard, Copenhagen, 1946, 186p.

8. Nørgaard, O., *Acta Path. Microbiol. Scand.*, 1950, v27, 811.

9. ———, *ibid.*, 1950, v27, 865.

10. ———, *ibid.*, 1954, v34, 336.

11. ———, *ibid.*, 1955, v36, 71.

12. Coons, A. H., and Kaplan, M. H., *J. Exp. Med.*, 1950, v91, 1.

13. Coons, A. H., Leduc, E. H., and Connolly, J. M., *ibid.*, 1955, v102, 49.

14. Casals, J., and Palacios, R., *ibid.*, 1941, v74, 409.

15. Hoyle, L., *Mon. Bull. Minist. Hlth. Lab. Serv.*, 1948, v7, 114.

16. Whitney, E., and Gnesh, G. M., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 356.

17. Whitney, E., Kraft, L. M., Lawson, W. R., and Gordon, I., *Bact. Proc.*, 1953, p50.

18. Wadsworth, A., *Standard Methods of the Division of Laboratories and Research of the New York State Department of Health,* 3d ed., Williams and Wilkins, Baltimore, Md., 1947, pp361-465.

19. Silverstein, A. M., *J. Immunol.*, 1954, v72, 187.

20. Gnesh, G. M., and Rapp, F., unpublished data.

Received July 28, 1955. P.S.E.B.M., 1955, v90.

Inhibition of Development of Chick Embryo Liver by Aminoguanidine. (22027)

ROBERT E. NEUMAN AND THOMAS A. MCCOY.* (Introduced by S. H. Wender.)

Biomedical Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, Okla.

Studies have been made on the nutrition, metabolism, and development of the chick embryo by injection of biologically active substances into the embryonated egg. Profound inhibition of growth with production of developmental abnormalities result from injection of certain substances(1-5), and Karnofsky(6) pointed out the value of this technic as a screening method for specific growth-inhibitors.

By extension of this technic to new classes of chemical compounds, we have observed a severe inhibition of the development of the chick embryo liver by aminoguanidine. This inhibition is rather specific and has no obvious effect on the other organs of the body under the conditions described.

* The authors gratefully acknowledge the technical assistance of Mr. Gene Conway and Mr. Merle Maxwell.

Methods. Aminoguanidine sulfate (AGS)† was dissolved in double distilled water to form

TABLE I. Effect of Aminoguanidine Sulfate on Mortality, Body and Liver Weight of Chick Embryo.*

Quantity injected (mg/egg)	No. of eggs	Embryos survived	Body wt (g)†	Liver wt (mg)†
H_2O	4	4	9.89 ± .89	173 ± 26
2	14	12	8.26 ± .96	139 ± 38
5	8	7	8.58 ± .52	66 ± 39
10	14	8	8.07 ± .69	74 ± 44
15	8	8	9.26 ± .71	65 ± 62
20	14	9	7.11 ± .79	45 ± 36‡
25	8	1	6.75 ± —	— —
40-60	9	0	— —	— —

* Yolk sac injections were made on 4-day-old embryos which were harvested 10 days later.

† Mean value ± avg dev.

‡ Six of the livers at this dosage each weighed less than 25 mg.

† Obtained from Distillation Products Industries, Eastman Organic Chemicals Department.

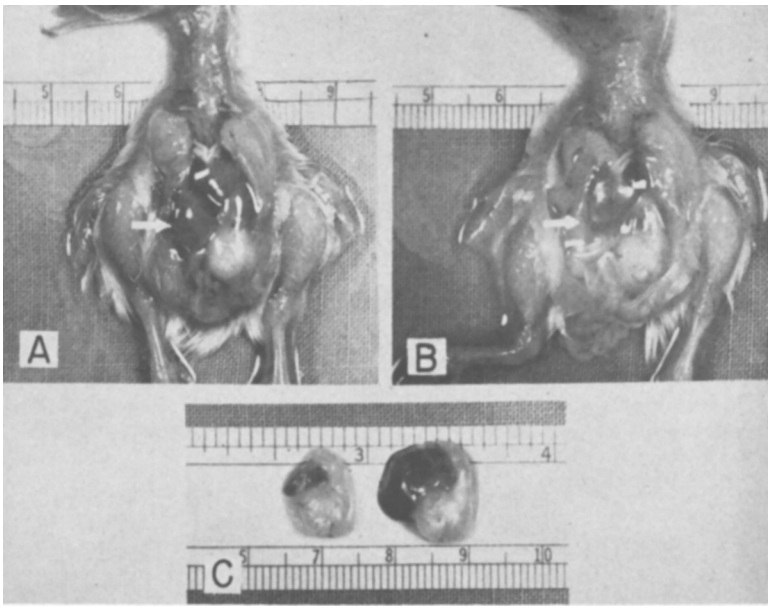

FIG. 1. Gross appearance of 14-day-old chick embryo after injection of 20 mg AGS at the 4th day of incubation. A—Control; B—Treated; C—Comparative size of livers.

a solution of desired maximal concentration with an adjusted pH of 5.6. The solution was sterilized by passage through a Selas filter of 03 porosity. Sterile double distilled water was used for further dilutions. White Leghorn eggs were incubated at 38°C and 80% humidity for 4 days. At the end of this time .4 ml of the solution to be tested was injected into the yolk according to conventional methods. After further incubation (usually until 14 days of age), the embryos were removed and examined. The specimens were preserved in 10% formalin. When histological examination was desired, the embryos of specific organs were embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

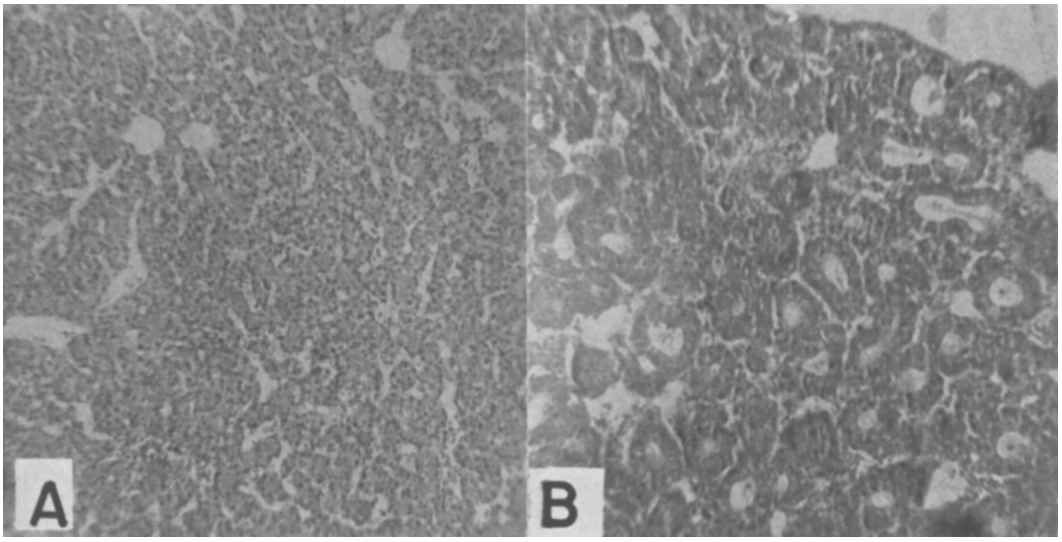

FIG. 2. Section of liver of 14-day-old chick embryo when injected at 4 days (hematoxylin eosin stain, 100 ×). A—Control; B—20 mg of AGS.

TABLE II. Effects of Aminoguanidine Sulfate on Chick Embryo when Administered at Different Embryonic Ages.*

Age at injection (days)	No. of eggs	Embryos survived	Body wt (g)†	Liver wt (mg)†
		H_2O injected		
0- 3	24	17	8.20 ± .66	155 ± 11
4-10	30	26	8.87 ± .74	175 ± 16
		2 mg aminoguanidine sulfate injected		
0	8	5	7.43 ±1.18	157 ± 11
2	8	4	— —	131 ± 25
3	6	4	8.01 ±1.89	75 ± 27
4	14	12	8.26 ± .96	139 ± 38
5	6	6	9.15 ± .96	169 ± 25
6	6	6	7.64 ± .45	161 ± 27
7	6	6	8.44 ± .73	177 ± 17
8	6	5	9.45 ± .60	195 ± 18
9	6	4	9.00 ± .70	180 ± 28
		10 mg aminoguanidine sulfate injected		
2	8	1	— —	5 —
3	6	4	7.96 ± .23	49 ± 13
4	14	8	8.07 ± .69	74 ± 44
5	6	6	8.99 ± .30	56 ± 33
6	6	5	7.70 ± .45	76 ± 19
7	6	2	7.27 ± .20	88 ± 25
8	5	2	9.00 ± .41	166 ± 25
9	6	5	8.00 ± .91	159 ± 9
		20 mg aminoguanidine sulfate injected		
2	6	—	— —	— —
3	6	2	7.32 ± .03	20 ± 3
4	14	9	7.11 ± .79	45 ± 36‡
5	12	8	7.74 ±1.02	48 ± 20
6	12	9	8.17 ± .57	95 ± 26
7	12	9	7.44 ± .41	97 ± 23
8	6	5	9.01 ± .77	141 ± 24
9	12	8	8.93 ± .79	165 ± 25
10	6	3	10.00 ± .66	182 ± 9

* Each dose was administered in 0.4 ml of solution injected into yolk. Embryos were examined at 14 days of incubation.

† Mean value ± avg dev.

‡ Six of the livers at this dosage each weighed less than 25 mg.

Results. The effect of gradated amounts of AGS on the mortality, body weight and liver mass of the chick embryo examined at 14 days of age is recorded in Table I. The number of embryos surviving 25 mg of AGS was negligible. Although the body weight at lower dosage levels was not greatly depressed, the liver mass underwent a marked reduction. At the 20 mg dosage level, ⅔ of the livers weighed less than 25 mg each and several of these consisted of only traces of tissue adherent to the duodenum. The relative mass of livers in typical treated and untreated embryos, as it appeared on gross examination, is shown in Fig. 1. The decrease in liver size and the presence of liver lesions were evident as low as 2 mg AGS dosage levels The other organs were relatively normal in gross appearance and histological sections.

The effects of 3 concentrations of AGS administered at embryonic ages varying from 0-10 days were determined (Table II). The survival rate of embryos injected prior to 4 days incubation was quite low; nevertheless, data indicate the liver to be most susceptible to inhibition when treatments are at 2-4 days. As the age of injection increased, the liver mass is decreasingly affected by a given dosage level of AGS. Finally, the liver appears perfectly normal following injection at 9-10 days of age.

In order to observe the progressive effects of AGS on the liver, eggs injected with 20 mg AGS at 4 days were removed at subsequent 48-hour intervals. Histological examination revealed that cord formation is retarded within 96 hours. In the typical liver of a treated 14-day embryo, the cord development was depressed and the parenchymal cells were replaced in large part by connective tissue and tubule-like structures (Fig. 2). Examination of serial sections revealed that these latter structures were actually oval or rounded cysts. Livers of embryos surviving beyond 14 days appeared to resume normal growth.

Discussion. Aminoguanidine has been tested on a variety of tumors and the results were considered negative(7-10). Brochman *et al.* (11) noted significant tumor inhibition by acid hydrazides such as diaminobiuret, isonicotinic hydrazide, and substituted benzoic acid hydrazides when mice bearing Sarcoma 180 were maintained on vit. B_6-deficient diet. This effect was not observed on a complete diet, but an increased restriction of tumor growth resulted when the acid hydrazides were administered in combination with desoxypyridoxine on mice maintained on a B_6-deficient diet. In the present reported studies, when pyridoxal or pyridoxine was administered in 2 mg/egg quantities concurrently with 2-4 mg/egg of AGS, no effect on the liver of the embryo was noted. Guanidine hydrochloride injected into the yolk of a 4-day embryonated egg killed all specimens at the 15

mg/egg level. Smaller quantities produced some minor liver damage but no particular abnormality as was noted in the case of AGS. An inquiry is now being made into the effect of other hydrazides upon the chick embryo. In general, these are lethal in mg doses and produce minor liver damage at lower levels. In no instance have other hydrazides produced the extreme inhibition of the liver as reported here.

Summary. Aminoguanidine sulfate when injected into the yolk of a 4-day embryonated egg produced a severe inhibition of liver development of the chick embryo when the specimens were examined 10 days later. Apparently, the parenchymal cells were the sites of inhibition as the surviving tissue, in addition to being reduced in mass, was composed largely of connective tissue and cystic structures. Other organs and tissues of the specimens appeared to be relatively unaffected. The most effective sub-lethal dose at 4 days incubation was ineffective when administered at 9-10 days of incubation. Other hydrazides possess peculiar effects which bear no resemblance to that of AGS.

1. Karnofsky, D. A., Ridgway, L. P., and Patterson, P. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 447.

2. Karnofsky, D. A., Dagg, C. P., and Lacon, C. R., *Fed. Proc.*, 1955, v14, 439.

3. Ridgway, L. P., and Karnofsky, D. A., *Ann. N. Y. Acad. Sci.*, 1952, v55, 203.

4. Karnofsky, D. A., Ridgway, L. P., and Patterson, P. A., *Endocrinology*, 1951, v48, 586.

5. Landauer, W., *J. Cell. Comp. Physiol.*, 1954, v43, 261.

6. Karnofsky, D. A., *Cancer Research*, 1955, Supp. 3, 83.

7. Field, J. B., Boryezka, A., and Corta, F., *ibid.*, 1955, Supp. 2,3.

8. Skipper, H. E., and Thomson, J. R., *ibid.*, 1955, Supp. 2, 147.

9. Stock, C. C., Clarke, D. A., Phillips, F. S., and Barclay, R. K., *ibid.*, 1955, Supp. 2, 179.

10. Turner, F. C., *ibid.*, 1955, Supp. 2, 339.

11. Brochman, R. W., Schabel, F. M., Jr., Thomson, J. R., and Skipper, H. E., *Fed. Proc.*, 1955, v14, 186.

Received July 28, 1955. P.S.E.B.M., 1955, v90.

Development of Hypertension and Cardiovascular-Renal Lesions During Adrenal Regeneration in the Rat.* (22028)

FLOYD R. SKELTON. (Introduced by D. J. Ingle.)

Department of Pathology, Medical College of Georgia, Augusta.

During a study of the effect of bilateral adrenalectomy on the production of hypertension and cardiovascular-renal lesions by methylandrostenediol in the rat it was observed that one control animal developed hypertension. Autopsy showed that the right adrenal of this rat had been completely removed but that a large regenerated adrenal cortex was present on the left side attached to the adrenalectomy scar. The possibility came to mind that the hypertension in this animal may have been the result of excessive hormonal secretion by the regenerating adrenal tissue. The following experiment was performed to test this hypothesis.

Methods. Twenty-four female Sprague-Dawley rats averaging 62 g body weight were unilaterally nephrectomized and given a diet of Purina Laboratory chow and 1% sodium chloride solution *ad libitum*. Twelve rats served as controls and 12 were unilaterally adrenalectomized with contra-lateral adrenal enucleation(1). This procedure consists of a small incision in capsule of the gland through which the bulk of glandular tissue is extruded by gentle application of pressure with curved forceps. The capsule and some adherent cells of the zona glomerulosa are

* This investigation was supported by a research grant (H-2002) from the National Heart Institute, of the National Institutes of Health, Public Health Service.