integration of normoblasts, change of nuclear pattern in erythroid elements and megaloblasts. Doses given were lethal and animals succumbed with anorexia, weight loss, dehydration, and hemorrhagic diarrhea following severe ileitis and ulcerative colitis. It is suggested that the syndrome results from antagonism of folic acid.

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17233. Chronic Toxicity of Gamma Isomer of Hexachlorocyclohexane in the Albino Rat.

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Included in the report by Slade[1] on the discovery of the potent insecticide, "Gammexane," the gamma isomer of benzene hexachloride (γ-BHC)* were investigations of the acute and chronic toxicity of the drug in laboratory rats. His report indicates that the gamma isomer is much more toxic than the α, β, or δ isomers on oral administration, but, when mixed with the diet, levels of γ-BHC up to 30 mg per rat per day had no effect over a 5 week period. Other acute experiments have demonstrated by oral, subcutaneous and intravenous administration, the toxicity of γ-BHC,[2,3] while Laug[4] reported that the gamma isomer was non-toxic when fed in the diet at 20, 500, and 1000 p.p.m. for periods up to 114 days.

Other workers have been interested in the anti-inositol properties of the gamma isomer, since it was reported to resemble the structure of inositol.[1] Their results indicate that the inhibitory effect of γ-BHC is reversed by inositol in the growth of a microorganism,[5] in the α-amylase enzyme system of the pancreas,[6] and in the growing onion root.[7]

A study of transmethylation and lipotropic agents stimulated in this laboratory by the work of Griffith indicated that it might be of interest to examine the anti-inositol and toxic properties of the gamma isomer in the rat, possibly to make available another method of studying inositol metabolism in the intact animal.

Weanling male rats of the St. Louis University strain, 21-24 days old, weighing 44-49 g, were housed in screen cages and allowed food and water *ad libitum*. The animals were weighed twice weekly. The diet to which supplements of γ-BHC and inositol were added was as follows: Casein, 15.0; l-cystine, 0.3; salts 3A,† 2.0; salts 3B,‡ 2.0; celluration, 2.0; B-complex mixture,§ 1.0; choline chlor-

1 Slade, R. E., *Chem. Ind.*, 1945, **64**, 314.

* The term, gamma benzene hexachloride, has been approved by the Committee on Insecticide Nomenclature as the accepted name for the gamma isomer of hexachlorocyclohexane (*Science*, 1949, **109**, 330).

2 Cameron, G. R., and Burgess, F., Insecticide Development Panel Report (44), 1944, 131.

3 McNamara, B. P., and Krop, S., *J. Pharm. Exp. Therap.*, 1948, **92**, 140.

4 Laug, E. P., *J. Pharm. Exp. Therap.*, 1948, **93**, 277.

5 Kirkwood, S., and Phillips, P. H., *J. Biol. Chem.*, 1946, **163**, 251.

6 Lane, R. L., and Williams, R. J., *Arch. Biochem.*, 1948, **19**, 329.

7 Chargaff, E., Stewart, R. N., and Magasanik, B., *Science*, 1948, **108**, 556.

† The salt and vitamin mixtures were devised and thoroughly tested by Dr. Wendell H. Griffith. Since the compositions of these supplements have not been published, the authors wish to express their thanks to Dr. Griffith for his permission to present them here. Salts 3A: $CuSO_4$, 0.5; $CoSO_4 \cdot 7\ H_2O$, 0.1; $ZnSO_4 \cdot 7\ H_2O$, 0.9; $MnSO_4 \cdot 4\ H_2O$, 4.0; $K_2Al_2(SO_4)_3 \cdot 24\ H_2O$, 0.07; NaF, 0.05; Ferric citrate, 24.38; $CaCO_3$, 100; Ca citrate, 185; $MgCO_3$, 45.0; K_2HPO_4, 140 g.

‡ Salts 3B: KI, 0.8; NaCl, 169.2; K_2HPO_4, 100; $CaHPO_4$, 230 g.

TABLE I.
Mortality and Growth Rates of Rats Receiving γ-BHC.

Supplement				
Inositol, mg/g	γ-BHC, mg/g	No. rats	% mortality	Growth, g/wk (avg)
—	—	24	0	28.0
0.5	—	12	0	26.6
2.0	—	8	0	28.9
0	0.8	12	75	11.9
0.5	0.8	24	63	17.1
2.0	0.8	18	72	18.2
0	0.4	16	6	26.0
0.5	0.4	22	36	27.4
2.0	0.4	24	8	28.2

TABLE II.
Mortality and Growth Rates of Littermate Rats Receiving γ-BHC.

	Supplement				Growth rate, g gain/wk		
Group	Inositol, mg/g	γ-BHC, mg/g	No. rats	% mortality	1	2	Avg 4 wks
1	—	—	22	0	24.3	29.6	30.1
2	0.5	—	22	0	25.9	27.6	30.1
3	2.0	—	22	0	24.8	30.0	29.8
4	4.0	—	22	0	26.6	29.8	31.3
5	—	0.4	22	14	10.6	27.6	25.4
6	0.5	0.4	22	14	14.1	29.8	27.9
7	2.0	0.4	22	18	11.6	29.4	27.1
8	4.0	0.4	22	36	15.2	29.7	28.7

ide, 0.4; fat-soluble vitamin mixture,‖ 2.0; lard, 8.0; sucrose, 67.3%.

Supplements of γ-BHC were dissolved in melted lard, while inositol was added at the expense of sucrose. Four week feeding periods were studied throughout. Results of preliminary experiments are presented in Table I.

Control animals on the suboptimal (15%) protein levels gained weight at a rate of 26.6-28.9 g per week. Since the animals receiving no dietary inositol grew as well as those receiving adequate quantities of inositol, it may be stated that with these rations this strain of rat requires no dietary inositol. The presence of γ-BHC in these diets led to markedly diminished growth rates and significant mortality rates, particularly during the first two weeks. Addition of inositol seemed to be without effect. No alopecia, spectacled eye, or other possible signs of inositol deficiency were observed in animals maintained on inositol-free diets for experimental periods up to 8 weeks.

The sequence of events following the administration of sufficient γ-BHC is striking; within 24-48 hours the temperament of the animals alters from docility to ferocity; they react explosively to tactile stimuli, leaping, squealing, and biting as if in pain when touched lightly on the back. They seem to be less sensitive to auditory stimuli, but, superficially, the picture resembles somewhat that seen in magnesium deficiency.[8] Generalized tonic-clonic convulsions are frequent,

§ B-Complex mixture: Ca pantothenate, 5.0; niacinamide, 2.5; para-aminobenzoic acid, 1.0; riboflavin, 1.0; pyridoxine • HCl, 0.5; thiamine • HCl, 0.5; biotin, 0.1; "Folvite," 0.05; and sucrose, 989.35 g.

‖ Fat-soluble vitamin mixture: Peanut oil, 195.0; α-tocopherol acetate, 5.0 g. Forty g of this solution is added to cod liver oil, 2.0; corn oil (Mazola), 157.5; 2-methyl-1,4-naphthoquinone, 0.5 g.

[8] Kruse, H. D., Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1932, **96**, 519.

accompanied by hyperpnea and later by hypopnea and exhaustion (some aspects of this syndrome were observed in acute toxicity experiments with γ-BHC[2,3]). Some animals having 2 or more convulsions during the first week have subsequently recovered completely. Of weanling animals failing to survive, death occurred from the second to tenth experimental days.

Additional experiments examined the toxic effects of levels of dietary γ-BHC at 0.6 and 0.2 mg per gram of food. The higher level was lethal to a large proportion of animals, while the lower level was non-toxic and without effect on growth rates. Thus the optimal level of γ-BHC for study was selected at 0.4 mg/g of food; in a similar experiment litter mate controls were used throughout the groups. Growth rates and mortality data are presented in Table II.

The data again demonstrate that this strain of rat does not require supplemental inositol under these experimental conditions. The addition of 0.4 mg of γ-BHC to the diets reduced growth rates markedly during the first week, but during the succeeding 3 weeks, growth rates of surviving animals receiving γ-BHC compared favorably with those of the controls, indicating the development of tolerance. The addition of increasing amounts of inositol seemed to combat slightly the growth depressant effect of γ-BHC (group 5 compared with groups 6, 7 and 8), but had also the effect of increasing mortality. The latter may have been due to a slight increase in food consumption during the first week with a slightly greater intake of γ-BHC in those animals receiving higher levels of inositol. (The first week the controls averaged 7.2 g of food per rat per day, while the food intakes of the animals receiving γ-BHC were: group 5, 4.9; group 6, 5.1; group 7, 5.1; and group 8, 5.3 g of food per rat per day.)

In other experiments the effect of 0.8 mg γ-BHC per gram of food was tested in weanling rats fed a stock laboratory ration, *i.e.*, ground Purina lab chow, to ascertain if the type of diet influenced the reaction to the drug. The young animals exhibited hyperirritability, hyperactivity, convulsions, retarded growth, and a mortality of 25%. Adult rats raised on chow, when placed on the basal ration plus γ-BHC at the 0.8 mg level, lost 10% of their body weight over 10-14 days; in the succeeding two weeks they returned to their starting weights and continued a slow gain. Irritability and infrequent convulsions without mortality resulted.

Animals raised on the highly purified ration (inositol-free) to body weights of 200-300 grams, showed typical symptomatology with a mortality of 50% when the gamma isomer was added to the ration at the level of 0.8 mg per g of food. These animals obtained 3.4-4.1 mg of γ-BHC per rat per day while the weanling rats dying (Table II) received between 1.8-3.1 mg. (Adult rats dying ate 188 mg/kg over a 10-day average survival time, while weanling rats consumed 200 mg/kg body weight over a 4-day average survival period.)

These results are not in accord with those of Slade[1] and Laug[4] whose experimental diets were not reported; Slade reported γ-BHC to be non-toxic when mixed with the diet in amounts up to 30 mg per rat per day, while calculation of the data presented by Laug reveals no toxic effects with dosages up to 20 mg per day. Recently Fitzhugh *et al.*[9] reported that the gamma isomer, when mixed with the diet, is toxic at the level of 0.8 mg per gram of food.

The authors wish to point out that stress was placed upon the rats used in these experiments by suboptimal dietary protein levels in an effort to enhance the action of γ-BHC. Further, animals raised on stock rations tolerated diets containing γ-BHC much better than those raised on experimental diets. These discrepancies may in part be due to the higher nutritive value of normal stock rations, as compared with highly purified rations.

A possible explanation of the failure of inositol to reverse the chronic toxic effects of γ-BHC may be the absence of a demonstrable inositol deficiency in our experiments using rats. A similar failure to demonstrate protection by massive doses of inositol in acute toxicity studies in rabbits has been reported by McNamara and Krop.[10] A recent report

[9] Fitzhugh, O. G., Nelson, A. A., and Holland, O. L., *Fed. Proc.*, 1949, **8**, 291.

by Bijvoet[11] which appeared after our experiments were well under way, indicates lack of similarity in spatial configuration between inositol and γ-BHC. Thus, if this dissimilarity in structure is confirmed, the counteraction of γ-BHC by inositol, previously reported,[5,6,7] may not be the result of specific structural antagonism.

The mechanism of action of γ-BHC is still obscure; after the demonstration by Lane and Williams[6] that inositol is a constituent of α-amylase, the activity of which is inhibited by the gamma isomer, the possibility that hypoglycemia might be involved in the etiology of the convulsions was entertained. Blood sugar levels were determined in a limited series of control and γ-BHC-treated animals and were found within the normal range, even shortly before and after a convulsion.

Gross and microscopic examinations of animals dying after the administration of γ-BHC were performed by Drs. Vincente Moragues and Henry Pinkerton of the Department of Pathology at this institution. No significant pathology was noted. The authors wish to extend their thanks to them.

Conclusion. Gamma benzene hexachloride, mixed with highly purified rations, is toxic to the weanling and adult albino rat. The addition of inositol does not alleviate the toxic symptomatology of γ-BHC.

We wish to thank Mr. Jerome Martin of Commercial Solvents Corporation for his generous gift of γ-BHC, the melting point of which was 112-113°C (uncorr.), slightly higher than the melting points of 4 other samples from various sources.

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[10] McNamara, B. P., and Krop, S., *J. Pharm. Exp. Therap.*, 1948, **92**, 147.

[11] Bijvoet, J. M., *Rec. Trav. Chim.*, 1948, **67**, 777.

17234. Insulin Stimulation of Glycogen Formation in Rat Abdominal Muscle.*

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Gemmill[1] first demonstrated that insulin stimulated glycogen deposition from glucose in isolated rat diaphragm. Subsequent study with this tissue has disclosed the following important points. Low potassium[2] and high glucose[1] concentrations favor glycogen synthesis. The insulin effect can be demonstrated in diaphragm from either adrenalectomized or hypophysectomized[3] rats and so apparently is independent of the action of hormones from these sources. Desoxycorticosterone, corticosterone and some related substances give reduced glycogen levels when incubated with diaphragm muscle, the effect appearing to be of a glycogenolytic nature and separate from the glycogenic response to insulin.[4]

We have recently examined glucose metabolism in the rat diaphragm with the help of radioactive C^{14} glucose.[5] An increase in the radioactivity of the glycogen in the presence of insulin closely proportionate to the increased glycogen content by analysis provided additional evidence that glucose was the direct precursor of the glycogen rather than a stimulant of glycogenesis from other sources. Insulin pro-

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[1] Gemmill, C. L., *Bull. Johns Hopkins Hosp.*, 1940, **66**, 232; 1941, **68**, 329.

[2] Stadie, W. C., and Zapp, J. A., Jr., *J. Biol. Chem.*, 1947, **170**, 55.

[3] Perlmutter, M., and Greep, R. O., *J. Biol. Chem.*, 1948, **174**, 915.

[4] Verzar, F., and Wenner, V., *Biochem. J.*, 1948, **42**, 35, 48.

[5] Bartlett, G. R., Wick, A. N., and MacKay, E. M., *J. Biol. Chem.*, 1949, **178**, 1003.