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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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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TABLE I.
Effect of Insulin and Desoxycorticosterone on Glycogen in Isolated Rat Abdominal Muscle.

		Mg of glycogen per 100 mg (wet wt) muscle				
			Incubated 2 hr at 38° under O_2			
Exp.	Days fasted	Initial value	Without glucose	Glucose	Glucose + insulin	Glucose + DOC
1	0	.12	.10	.29	.50	.16
2	0	.16	.19	.35	.54	.30
3	0	.22	.13	.33	.42	.18
4	0	.39	.32	.52	.62	.35
5	0	.22	.21	.37	.59	.36
6	0	.28	.16	.40	.50	.22
7	1	.21	.14	.29	.52	.21
8	1	.15	.16	.32	.38	.17
9	1	.24	.15	.44	.65	.24
10	2	.12	.16	.30	.48	.16
11	2	.21	.14	.32	.45	.20
12	3	.25	.10	.26	.44	.23
		—	—	—	—	—
Avg		.21	.16	.35	.51	.23

duced a small but significant increase in the radioactive CO_2, hence an acceleration of the combustion of the sugar. Desoxycorticosterone and corticosterone antagonized glycogen formation without appreciably influencing glucose utilization or respiration values and without increasing radioactive carbon dioxide.

The use of insulin in general with isolated tissue slice, mince or extracts, has proved discouraging. Few verifiable effects of any magnitude have been published other than the rat diaphragm work. There has been considerable interest in the reports from Cori's laboratory suggesting that insulin can release some sort of combined pituitary-adrenal cortical inhibition of hexokinase,[6,7] the enzyme responsible for the first step in the utilization of glucose; and unified hypotheses covering the hexokinase action have been offered to explain insulin action *in vivo*.[8] Unfortunately the hexokinase experiments have been ambiguous and difficult to repeat and there appears to be some doubt as to whether even one of the points of action of insulin is a hexokinase inhibitory release.[9,10]

Diaphragm muscle is an important tissue but a specialized muscle with a limited resting period and undergoing continuous rhythmic contractions throughout life. On exploring the possibility of using tissue other than the diaphragm for insulin effects, we have generally found that chopped, shredded or teased muscle loses its ability to synthesize glycogen from glucose. However, we have been able to dissect the internal oblique abdominal muscle of the rat with a minimum of tearing and find that this muscle *in vitro* will give considerable glycogen synthesis from added glucose and also a further large increase of glycogen in the presence of very small amounts of insulin. As in the case of the diaphragm less glycogen is found in the presence of desoxycorticosterone. Results of experiments with this tissue are recorded in Table I. 400 - 500 mg wet weight of abdominal muscle was incubated in 3.0 ml of pH 7.4 Ringer phosphate with or without 0.2 molar glucose, 0.1 unit per ml of insulin and 10 μg per ml of desoxycorticosterone.† Flasks were shaken for 2 hours in an oxygen atmosphere. Glycogen was assayed by the usual alkali digestion-alcohol precipitation

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† Pure Zn insulin was obtained through the courtesy of Dr. Edwin E. Hays of Armour and Co., and desoxycorticosterone from Dr. Edward Henderson of the Schering Corp.

method.[11] 100 - 120 g female albino rats were used and that portion of each internal oblique muscle which could be separated as a thin layer without appreciable trauma was placed intact in the incubating flask. When the muscle was cut into a series of thin strips, little or no glycogen synthesis took place.

The results reported here are evidence that insulin-glycogenesis stimulation *in vitro* is not peculiar to diaphragm muscle. New material is available for further investigation of the action of insulin and other factors controlling glycogen formation and utilization.

Summary. If used in thin sheets with a minimum of tissue injury isolated skeletal muscle in the form of the internal oblique muscle of the rat forms more glycogen under the influence of insulin just as does the specialized muscle comprising the diaphragm. Desoxycorticosterone reduces the amount of glycogen formed by incubation with glucose alone as in the case of diaphragm muscle. Skeletal muscle offers new material for further investigation of the action of insulin.

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17235. Normal and Seizure Levels of Lactate, Pyruvate and Acid-Soluble Phosphates in the Cerebellum and Cerebrum.*

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Seizures induced by various means have been shown to result in an increase in lactic acid, pyruvic acid and inorganic phosphorus and a decrease in high energy phosphate levels in the cerebral cortex[1,2] or whole brain[3] of various experimental animals. There is little data on the several parts of the brain. As reported previously it is possible to show that seizures induced by low doses of nitrogen mustards start first in the cerebellum; further, the convulsive activity of these compounds is potentiated by inhalation of carbon dioxide.[4] Inhalation of carbon dioxide tends to prevent the changes in lactic acid and phosphate attendant upon seizures.[5] As a sequel to the above studies, seizures were induced in a series of cats using representative nitrogen mustards and various other convulsants, the brains were frozen *in situ* and the cerebrum and cerebellum then analyzed separately for lactic acid, pyruvic acid, inorganic phosphorus, and high energy phosphates.

Experimental. Preparation of the animals, methods of EEG and EKG recording, procedure of freezing with liquid air and methods of analysis have been reported in detail in previous papers from this laboratory.[1,5] Seizures were induced by intravenous injections of the compounds indicated in Table I. Cerebral and cerebellar cortex were easily dissected from the frozen brains with a small chisel and mallet. During the dissection frequent applications of liquid air were used to maintain the tissue in a frozen state.

Results. The data are summarized in Table I. In normal brains frozen without seizures the levels of the constituents determined were very similar in both cerebellum and cerebrum and compared well with those previously reported[1,5] except that the phosphocreatine levels were somewhat low. In brains frozen during seizures, however, marked dif-

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