brown fat is poorly understood but an endocrine function has been suggested for it.[9]

Summary. During pregnancy and lactation the metrial gland is marked successively by the prominence of (1) ribonucleic acid, (2) eoinophilic granules and glycogen, and (3) lipids. These appear to be related, respectively, to growth and secretion, nutrition for the embryo and steroid metabolism.

[9] Long, J. A., *Anat. Rec.*, 1922, **23**, 107.

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Effect of Bacterial Endotoxins on Glycogen Synthesis.*

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It was found previously that the liver and muscle of rabbits which were poisoned with bacterial endotoxins showed a considerable depletion in glycogen.[1] Since the mechanism of this decrease in tissue glycogen was not known, further experiments were performed to investigate this problem. The experiments described in this paper deal with the effect of endotoxins on the synthetic process of glycogen formation from glucose and pyruvate *in vivo* and *in vitro*.

Experimental. The effect of Salmonella endotoxin on glycogen synthesis *in vivo* was studied in rats under the following experimental conditions:[2] Eighteen white rats, ranging in weight from 250 to 310 g, were starved for 48 hours. During this period the animals had free access to water. At the time of the experiment the animals were divided in 2 equal groups, one of which served as controls while the other was injected intraperitoneally with 2 ml (20 mg solid content) of Salmonella endotoxin per rat. It was found in preliminary experiments that this amount of endotoxin was lethal in 4-6 hours. One hour after injection of the endotoxin, 3 rats in each group were sacrificed and the liver glycogen determined.[3] At the same time, the remaining 12 rats were given 3 ml of saturated glucose solution by stomach tube. Two and 3 hours after the administration of glucose 3 animals in each group were sacrificed and their livers analyzed for glycogen. As indicated in Table I, the liver glycogen of the normal rats increased 6-8 fold in 2-3 hours, while the rats injected with endotoxin showed no appreciable increase. It is thought that this effect of the endotoxin is not a non-specific protein effect, since the injection of 20 mg bovine plasma protein per rat had no measurable effect on liver glycogen synthesis.

TABLE I.
Effect of Salmonella Endotoxin on Glycogen Synthesis in the Livers of Rats.

Time	Liver glycogen (mg/g) Normal	Poisoned*
Before glucose	1.74	0.18
	2.84	1.25
	2.21	0.97
2 hr after	16.2	0.39
	11.6	0.25
	15.8	0.36
3 " "	18.6	0.43
	16.9	0.44
	17.0	0.35

Each value obtained on one animal.
* Poisoned rats were injected with 2 ml (20 mg dry wt) of endotoxin one hour before glucose.

The effect of endotoxins on glycogen synthesis *in vitro* was studied in rabbit liver slices

* This investigation was undertaken and supported jointly by the U. S. Navy, Office of Naval Research, and the University of Chicago.

[1] Kun, E., and Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 221.

[2] Barron, E. S. G., personal communication.

[3] Unbreit, W. W., Burris, R. H., and Stauffer, J. F., *Manometric Techniques and Related Methods for the Study of Tissue Metabolism* (Burgess Publishing Company), 1946, p. 170.

and in rat diaphragm. Rabbits were starved for 24 hours, then sacrificed and the liver slices kept in ice cold, substrate-free medium for 30 minutes. Five ml of the K-Ca medium of Buchanan, Hastings and Nesbett[4] were measured into glass-stoppered centrifuge tubes containing 50 mg glucose or 10 mg lithium pyruvate as substrate and various amounts of endotoxins. Liver slices, weighing 250-350 mg each were placed in tubes containing substrate plus 5 ml of medium and aerated with 95% O_2 + 5% CO_2 for 3 minutes. The tubes were then closed with glass stoppers and shaken for 1 hour at 37°C. The reaction was then stopped by the addition of 5 ml 60% KOH and the tubes were heated in a boiling water bath for 15 minutes. The glycogen was determined as glucose.[3] In each experiment liver slices were shaken in substrate-free medium simultaneously with the experimental tubes. The differences in glycogen content of these blanks and the experimental (containing substrate) were taken as a measure of glycogen synthesis. As shown in Tables II and III, there was less glycogen formed in the presence of meningococcal endotoxin than in the controls.

Since Gemill[5] and Hamman,[6,7] later Stadie and Zapp,[8] and Krahl and Cori[9] had demonstrated that insulin increased the glucose uptake of the rat diaphragm *in vitro*, it was of interest to determine whether the action of insulin is affected by endotoxin and vice versa. In these experiments, the method described by Krahl and Cori[8] was employed. This technic allows the use of pieces of the same diaphragm for experimental and control observations. Each experiment shown in Table IV gives the results obtained from strips of the same rat diaphragm. Insulin increased the glucose uptake of the rat diaphragm while the decrease of glucose utilization in the presence of Salmonella endotoxin was less in the presence of insulin.

TABLE II.
Inhibition by Meningococcal Endotoxin of Glycogen Synthesis* from Glucose in Rabbit Liver Slices *in Vitro*.

No. of exp.	Normal, mg/g	Plus meningococcal endotoxin 0.1 mg/ml medium, mg/g	% inhibition
1	2.6	1.36	55
2	3.1	0.88	72
3	2.1	1.11	47
4	1.9	0.36	81
5	2.7	1.2	56
6	2.4	0.96	60

* Glycogen synthesis is expressed in terms of mg glycogen formed in one hour per g of liver in 5 ml K-Ca medium in presence of 50 mg glucose substrate.
Gas—O_2. Temperature—37°C.

TABLE III.
Inhibition by Meningococcal Endotoxin of Glycogen Synthesis* from Pyruvate in Rabbit Liver Slices.

No. of exp.	Liver, γ/g	Liver plus endotoxin, γ/g	% inhibition
1	805	530 (5 mg toxin)	32
2	1100	710 (5 " ")	35
3	990	480 (10 " ")	50
4	780	370 (10 " ")	52
5	970	510 (10 " ")	47

* Glycogen synthesis is expressed in terms of micrograms of glycogen per g of liver synthesized in one hour in presence of 10 mg Li-pyruvate in 5 ml K-Ca medium.
Gas—O_2. Temperature—37°C.

Discussion. In all experiments, endotoxins of meningococcus and *Salmonella aertrycke* caused an inhibition of glycogen formation from pyruvate and glucose. The most striking effect could be observed *in vivo*, where this synthetic process was completely inhibited. These observations make it probable that the depletion of liver and muscle glycogen in toxemic animals may be at least partially explained on this basis.

It is significant that Cross and Holmes[10] observed similar inhibition of glycogen synthesis by diphtheria toxin. However, this

[4] Buchanan, J. M., Hastings, A. B., and Nesbett, F. B., *J. Biol. Chem.*, 1942, **145**, 715.

[5] Gemill, C. L., *The Johns Hopkins Hosp. Bull.*, 1940, **66**, 232.

[6] Gemill, C. L., and Hamman, L., Jr., *The Johns Hopkins Hosp. Bull.*, 1941, **68**, 50.

[7] Gemill, C. L., and Hamman, L., Jr., *The Johns Hopkins Hosp. Bull.*, 1941, **68**, 329.

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

[9] Krahl, M. E., and Cori, F. C., *J. Biol. Chem.*, 1947, **170**, 607.

[10] Cross, M. C. A., and Holmes, E. G., *Brit. J. Exp. Path.*, 1937, **18**, 370.

TABLE IV.
Effect of Salmonella Endotoxin and Insulin on the Glucose Uptake of the Rat Diaphragm.

No. of exp.	Normal, mg/g	+Insulin 1 unit/ml, mg/g	+Toxin 0.1 mg/ml, mg/g	+Toxin-+insulin, mg/g
1	3.44	4.4	2.1	4.0
2	2.91	3.92	1.9	3.1
3	4.89	5.1	3.1	4.6
4	3.94	4.6	0.1	3.7
5	4.95	5.4	3.2	4.4
6	2.81	4.21	2.0	2.6
7	2.5	3.40	0.6	1.6
8	4.7	6.1	2.2	4.8
9	3.9	5.1	1.9	2.8

Glucose uptake is expressed in terms of mg glucose per g of tissue utilized in 2 hours.

similarity does not mean that the mode of action of diphtheria toxin is necessarily the same as that of the endotoxins. It was recently found that the phosphorylation of glucose was inhibited by meningococcal endotoxin.[11] This inhibition, together with the inhibition of the succinoxidase[1] might serve as an explanation of the inhibition of glycogen synthesis.

Summary. Intraperitoneal injection of Salmonella endotoxin completely inhibited *in vivo* glycogen synthesis from glucose in the liver of rats. In liver slices of rabbits, there was less glycogen formed *in vitro* from glucose and pyruvate in the presence of meningococcal endotoxin. The glucose uptake of rat diaphragm *in vitro* was decreased by Salmonella endotoxin and this inhibition of glucose utilization could be diminished by the addition of insulin.

[11] Kun, E., *J. Biol. Chem.*, 1948, **174**, 761.

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Influence of Crude Trypsin Inhibitor on Utilization of Hydrolyzed Protein.

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As a result of the reports of Ham and Sandstedt[1,2] on the presence of a trypsin inhibitor in raw soybeans, there has been considerable interest regarding the possible role that this trypsin inhibitor plays in altering the nutritive value of proteins. A relationship between the well known phenomenon of increased nutritive value of soybean protein resulting from heat treatment and the destruction of trypsin inhibitor has been suggested by several investigators. In fact, an excellent correlation between nutritive value and trypsin inhibitor content was observed in soybean preparations heated under varying conditions by Westfall and Hauge.[3] Melnick, Oser, and Weiss[4] observed that the relative rates of liberation of methionine, lysine and leucine as a result of tryptic digestion were different in raw soybeans and in soybeans in which the trypsin inhibitor had been inactivated by heat. They concluded from this study that the influence of the trypsin inhibitor on nutritive value of the soybean protein was due to an alteration in the time factors of availability of essential amino acids, particularly methionine, in the intestinal tract. Desikachar and De[5] have provided some evidence that does not support the conclusions of Melnick and his associates. They compared the digestibility and biological value of unmodified raw soybeans with papain-digested soybeans to which a water extract of soybeans containing

[1] Ham, W., and Sandstedt, R. M., *J. Biol. Chem.*, 1944, **154**, 505.

[2] Ham, W., Sandstedt, R. M., and Mussehl, F. E., *J. Biol. Chem.*, 1945, **161**, 635.

[3] Westfall, R. J., and Hauge, S. M., *J. Nutrition*, 1948, **35**, 379.

[4] Melnick, D., Oser, B. L., and Weiss, S., *Science*, 1946, **103**, 326.

[5] Desikachar, H. S. R., and De, S. S., *Science*, 1947, **106**, 422.