cornstarch 86.5%. They were given approximately 20% alcohol in place of drinking water and daily supplements of 100 μg of thiamin, 50 of riboflavin, 20 of pyridoxine, 50 of calcium pantothenate and 1 mg of nicotinic acid. Of 20 rats examined to date, all died between the 46th and 111th experimental days (average 83) and all showed moderate to marked liver cirrhosis on gross and histological examination. In a preventive experiment, 40 litter mates were given 20 mg of choline daily in the supplement or 0.7% of methionine in the diet or the dietary casein was raised from 4% to 30% at the expense of the cornstarch. Combinations of these changes were also employed. None of the rats sacrificed at the end of 90 or 140 experimental days or dying during the experiment showed any evidence of liver cirrhosis. Most of them are alive and apparently healthy, some after an experimental period as long as 6 months. The experiment is being continued to make certain that this represents a true prevention rather than a slowing of the cirrhotic process.

Since rats given diet number 545 and water but no alcohol have died with an indistinguishable liver pathology, it appears that the diet is here the essential factor.

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Production of Cytoplasmic Inclusions in Liver Cells of Rats Injected with Certain Proteins.

JULIA T. WELD, WILLIAM C. VON GLAHN AND LUCY C. MITCHELL.

From the Department of Pathology, College of Physicians and Surgeons, New York City.

In connection with studies on the effect of intravenous injection of rats with hemolytic streptococcus extract toxin[1] * (toxic serum extracts of hemolytic streptococci), we discovered that under certain conditions large cytoplasmic bodies appear within the liver cells. On further study, we found that other proteins also caused the development of similar bodies. This preliminary paper deals with the methods used for the production of this lesion and its description and is based on a study of 850 rats.

In Table I are presented: first, the substances that cause the lesion

1 Weld, J. T., *J. Exp. Med.*, 1934, **59**, 83.

* In this work the streptococcus extract toxin is referred to as "streptococcus toxin."

when injected intravenously; second, the percentage of body weight of the substance injected; third, the time elapsing between injection and autopsy. In Table II are presented the substances studied that, under the conditions of these experiments, have not produced inclusions.

The important factors in the production of the lesion are as follows:

Age of Animal. In order to obtain lesions regularly, rats should be at least 7 weeks old. When younger rats are used, the results are often negative.

TABLE I.
Proteins Producing Liver Inclusions when Injected Intravenously into Rats.

	Substances injected intravenously	% of body weight injected	Autopsy—Hour after injection
1	Streptococcus toxin (1) prepared with either horse serum or rat serum	1	2,4,5,18,24,48 or 56 hr
2	Normal sheep serum	1,2 or 3	Immediately, 10 or 30 min., 2,4,5,18, 24,48,56 hr
3	Sheep serum albumen or sheep serum globulin	2	4 hr
4	Normal horse serum into specifically sensitized rats (anaphylactic shock) (2)	1.5	3,7,15,25,46,48,56 min. 1,2,3,4,5,6,18,24,48 hr
5	Fresh egg albumen	1	4 hr
6	Crystallized egg albumen combined to R.-salt dye (3)*	1 or 1.5	4 hr
7	Sheep serum albumen combined to R.-salt dye*	1.5	4 hr

*We are indebted to Dr. H. Smetana for this preparation.

TABLE II.
Proteins That Have Not Produced Liver Inclusions when Injected Intravenously into Rats.

	Substance injected intravenously	% of body weight injected	Autopsy—Hour after injection
1	Normal horse serum	1, 2 or 3	2,4,5,18,24 hr
2	Normal rat serum	2	4 hr
3	Streptococcus toxin* (1) heated to 56° for one hour	2	4 hr
4	Horse serum extract of non-hemolytic streptococcus	3	4 hr

*Extract is non-hemolytic and non-toxic.

Amount of Substances Injected. In general, the number and size of the inclusions obtained appear to be directly proportionate to the percentage of body weight of inoculum. To produce numerous and large inclusions, 1 to 3% of the body weight of the substance should be injected.

Earliest Time After Injection of Appearance of the Inclusions. The time of the appearance of the inclusion after injection varies with the material injected. Thus, when streptococcus toxin was injected, the inclusions first appeared at 2 hours after injection but were absent in 30 minutes. (No rats were killed sooner after injection than this.)† With sheep serum (2% of body weight) numerous "early" inclusions were found in 9 of 10 rats within 10 minutes after injection and even in one rat killed immediately. In the anaphylactic rats[2] inclusions were usually present 1 hour after injection and in several rats in less than 1 hour. With the other substances used, we have data only on rats killed 4 hours or more after injection (Table I).

Progress of the Lesion. The bodies appear to be most conspicuous and well-defined when the rats are killed 4 to 5 hours after injection. After 18 to 24 hours they are fewer and less well outlined. At 48 hours they are scarce and stain faintly. Merely a few pale faded globules were found in one only of 5 rats killed 56 hours after the injection of 2% of sheep serum.

Effect of Dietary Deficiency on the Production of the Liver

TABLE III.
Staining Reactions of Inclusions in Hepatic Cells.

Stains	Early. Granular stage After injection of a. sheep serum; b. horse serum into specifically sensitized rats	Late. Homogeneous stage After injection of a. sheep serum; b. horse serum into specifically sensitized rats	Late. Homogeneous stage After injection of streptococcus toxin
Hematoxylin and Eosin	Pale red	Yellow occasionally red	Metallic red or yellow
Azan Carmine	Blue	Brilliant orange	Brilliant orange
Phosphotungstic Acid Hematoxylin	Yellow	Blue	Blue*
Giemsa	Pale red or purplish red	Yellow or purplish red	Red

*Some inclusions are yellow or muddy yellow, probably early stage.

† In this work all rats were killed with ether.

2 Weld, J. T., and Mitchell, L. C., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 168.

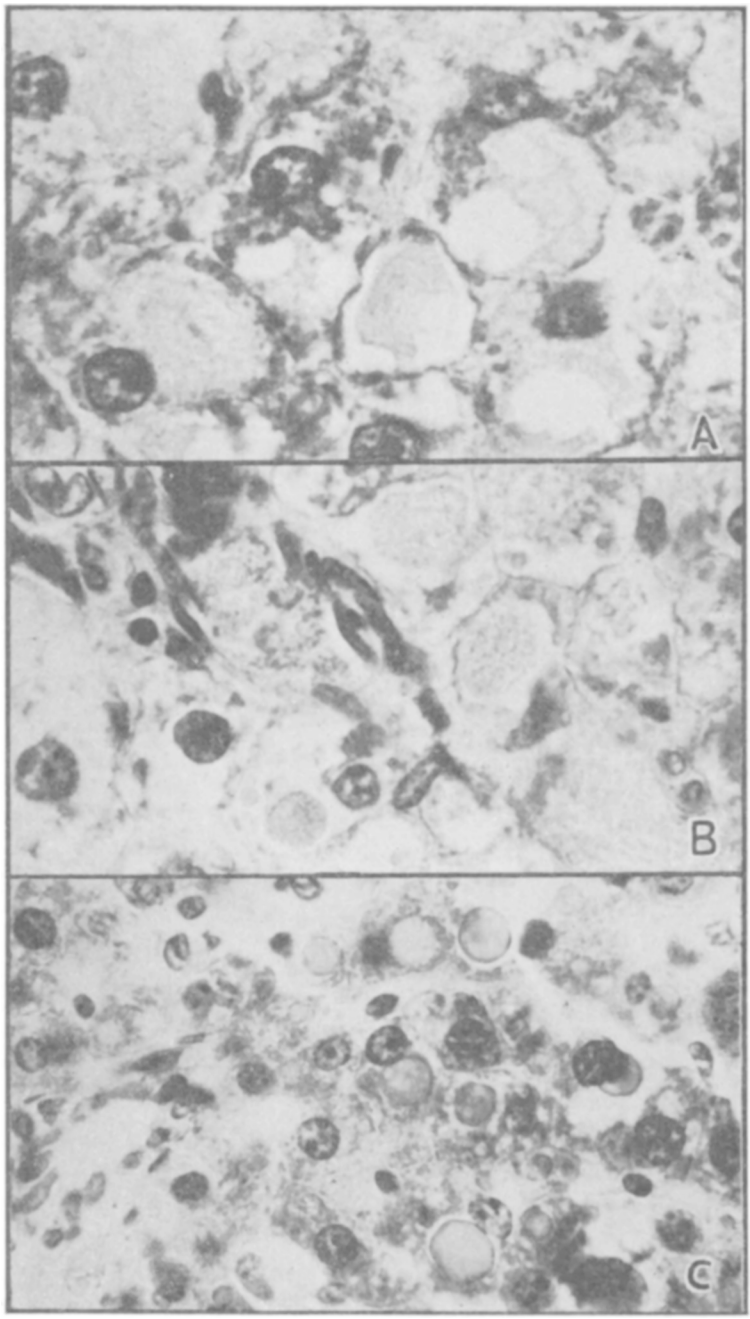

FIG. 1.

A. Liver of rat injected with streptococcus toxin. Killed 4 hours. × 500. Stained with hematoxylin and eosin.

B. Liver of rat sensitized to horse serum and reinjected with horse serum 13 days later (anaphylactic shock). Died 15 min. × 1050. Stained with hematoxylin and eosin.

C. Liver of rat sensitized to horse serum and reinjected with horse serum 13 days later (anaphylactic shock). Died 25 min. ×1050. Stained with hematoxylin and eosin.

Lesion. Except in the case of streptococcus toxin, no definite influence of dietary deficiency (essential amino acids, vitamin B complex or minerals) upon the formation of the bodies could be detected. Dietary deficiencies did seem to favor the development of the inclusions after injection of the streptococcus toxin.

Symptoms. All the substances that produce the liver lesions cause definite prostration lasting varying periods of time; and there appears to be a rough correspondence between the severity of the symptoms and the number of inclusion bodies. However, the bodies may persist after recovery from the prostration.

Description of the Inclusion Bodies. The cytoplasmic masses appear first as agglomerations of granules with fibrin within the liver cells (Fig. 1 A and B) that soon fuse into a roughly spherical or oblong homogeneous body (Fig. 1 C). This lies in a clear vacuole and often pushes the nucleus to one side. In unstained preparations they are greenish and have the same refractivity as erythrocytes. Their reaction to various stains is shown in Table III. Bodies of similar appearance are found also within the Kupffer cells, but they appear only after 2 hours and are smaller in size. Following injection of R-salt-protein,[3] the liver cell inclusions show a varying intensity of red staining; the Kupffer cells are filled with bright red particles. The inclusions do not stain as fat or glycogen.

The interesting problem, that we are studying further, is the chemical relation of these bodies in the liver to the injected protein.

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Demonstration of Myelolytic Substances in Disseminated Sclerosis.

ARTHUR WEIL AND GERT HEILBRUNN.

From the Institute of Neurology, Northwestern University, and the Elgin State Hospital.

Following the study of the myelolytic action of substances like saponin, bile salts and snake venoms upon the myelin sheaths of rat

[3] Heidelberger, Michael, and Kendall, Forrest E., *J. Exp. Med.*, 1933, **58**, 137.