Influence on Carbohydrate Metabolism of Experimentally Induced Hepatic Changes

IV. Block of the Reticulo-Endothelial System with Special Reference to the Kupffer Cell*

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NUMEROUS investigators have established the fact that the reticulo-endothelial system plays a part in the metabolism of fats and proteins. Comparatively little work has been done regarding the participation of this system in the metabolism of carbohydrates in spite of the fact that our knowledge regarding the fate of carbohydrates in the body is more advanced than that of other food elements. Several workers have studied the effect of reticulo-endothelial block on the blood-sugar level, but their results have been conflicting (1). Klein and Levinson (2), during continuous intravenous injections of India ink, observed an initial rise in blood-sugar, followed by a prolonged fall which they ascribed to exhaustion of available glycogen. Nakaya (3), from a study of hepatic glycogen after carbohydrate feedings before and after intravenous injection of India ink, concluded that the formation of glycogen is due largely to activity of the reticulo-endothelial system. Messina (4) performed extensive experiments showing reduced dextrose tolerance and decreased hyperglycemia after epinephrine in blocked rabbits. Unfortunately, the use of toxic blocking substances which caused marked loss of weight and eventually death in all animals rendered the significance of his data uncertain, especially since these findings are similar to those obtained by one of us (T.L.A.) after poisoning of the liver with phosphorus and with chloroform (5, 6).

Our investigation consisted of first depressing the function of the intravascular components of the reticulo-endothelial system by partial block, and then studying the blood-sugar level, the responses of the organism to "loads" imposed on the mechanisms dealing with assimilation and with mobilization of sugar, and the glycogen content of the liver and muscles.

METHODS

In blocking experiments, probably more than in any other biological research, the evaluation of the obtained data depends upon the readers' exact knowledge of the experimental conditions.

Animals. Jaffe (7) recommends rabbits as being among the animals most suited to blocking experiments. Accordingly healthy, growing rabbits weighing about 2 kg. were used in this work. They were kept on a diet consisting of rolled oats and alfalfa hay, with the addition of green cabbage leaves and carrots twice a week.

Blocking substances. Since our object was to produce physical block of the reticulo-endothelial system limited to its intravascular elements, the blocking substance of choice was a coarse, non-toxic, chemically inert, dispersed colloid which would not pass through endothelial walls, thus avoiding block of extravascular reticulo-endothelial cells or penetration into parenchymatous cells of glandular organs. During the early part of our work we used one part of Higgins' Black American India Ink diluted with two parts of distilled water as a substance thought to answer these requirements (8, 9). Later an article by Victor, Van Buren and Smith (10), dealing with the influence of India ink injections on the hepatic excretion of dyes, brought to our attention the fact that India ink may contain some toxic element, possibly camphor. Higgins Company refused our request to name the ingredients of their product, and therefore we repeated our experiments using separately the precipitated carbon and the carbon-free fractions of India ink, prepared according to directions received from Doctor H. P. Smith. Since eight rabbits injected with the resuspended carbon died from embolism following one or two injections, we substituted for carbon a suspension of colloidal graphite* in gum acacia. In preparation, the directions of Higgins and Murphy (11) were followed.

In attempting to reduce the functional capacity of the reticulo-endothelial system, the so-called Arndt-Schulze law must be taken into consideration. This repeatedly confirmed law postulates that small amounts of a blocking

*Hydro-Kollag "300" of E. de Haen Co.
substance stimulate, and large amounts depress, the function of the storing cells. Frequently errors are committed by giving an insufficient dose. From at least one study (12), thus far unconfirmed, it appears possible that an overdose may also increase the functional capacity of the reticulo-endothelium. We attempted to avoid both extremes by determining the effect of different amounts of the blocking substance.

Timing of experiments. Opinions differ as to the duration of effective block produced by single injections of a blocking substance. According to some authors (13), the effect of multiple injections may last as long as several weeks; others deny the existence of any cumulative effect with prolonged vital staining (7). In view of such disagreement, and because it is possible that different functions of the reticulo-endothelial system may manifest impairment for different periods of time, we tested our rabbits in separate experiments at intervals of 30 minutes, 5 hours, 16 hours and 24 hours following injection of the blocking substance.

Tests. In addition to single blood-sugar determinations, two procedures were employed: 1. The dextrose tolerance test, consisting of administration of 2.5 gm. of dextrose per kg. of body weight. The dextrose was given in a 15 per cent solution by stomach tube, and the blood-sugar level was determined at intervals of 30 min., 1 hour, 2 hours and 3 hours. 2. The blood sugar mobilization test, consisting of a subcutaneous injection of 0.05 mg. of epinephrine per kg. of body weight, which was followed by half-hourly blood-sugar determinations over a period of 2 hours.

To check the results obtained with these two tests, a modification of the dextrose tolerance test was used in which 2.5 gm. of dextrose per kg. were given by stomach in a 5 per cent solution, and 0.5 unit of insulin per kg. of rabbit were injected subcutaneously. Epinephrine was given at the end of 3 hours, and the blood-sugar was followed for 5 hours.

The rabbits were not fasted before the tests because, in our experience (14), fasting for even 16 hours affects the carbohydrate equilibrium of these animals.

Blood-sugar determinations were made in duplicate by the method of Hagedorn-Jensen. For glyogen determinations, the rabbits were sacrificed by a blow below the occiput. The entire livers and the adductor longus and adductor magnus muscles were immediately removed and placed in boiling potassium hydroxide after the method of Pfüger.

RESULTS

In the course of this work over 400 blood-sugar curves were obtained, and for brevity the following manner of presenting our data was chosen. For every curve the average blood-sugar was calculated. By subtracting the figure for the initial blood-sugar from that of the average blood-sugar, a single figure is obtained which expresses the result of the test in terms of disposal or of mobilization of sugar. For instance, the average of a blood-sugar curve obtained at 30 minute intervals after a injection of epinephrine and reading 92, 130, 138, 128 and 116 mg. per cent, is 125 mg. per cent. By subtracting the initial blood-sugar of 92 mg. from 125 mg., the result of plus 33 mg. is obtained. This figure expresses the degree of mobilization of sugar following injection of epinephrine in a given rabbit. In general, the peak of the curves paralleled this increase in the average blood-sugar, but was less reliable.

With this method of calculation, even small differences are significant because each figure represents an elevation of blood-sugar for the duration of the experiment. By averaging these figures from all rabbits for each day of the experiment, we can plot a curve that shows at a glance the effect of successive injections of a blocking substance on utilization or mobilization of sugar.

Experiments with India ink. 1. An initial injection of 10 c.c. of India ink was given each of 12 rabbits. This was followed by daily injections of 5 c.c. The animals were tested 16 hours after each injection. The average morning blood-sugar as seen in Chart I was lowered for a time, but eventually this effect of the injections wore off. During the dextrose tolerance test the increase in average blood-sugar at first diminished rapidly (see Chart II), but began to rise again after

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Average Increase of Sugar in Mg.

![Graph](chart1.png)

**Chart I.** Morning blood sugar of 12 rabbits during and of 6 rabbits after a period of daily India ink injections (5 c.c. given 16 hours previously).

Average Increase of Blood Sugar in Mg.

![Graph](chart2.png)

**Chart II.** Results of the dextrose tolerance and epinephrine tests in 12 rabbits receiving daily 5 c.c. of India ink. The tests were performed 16 hours after the injections.