Failure of Methyl Palmitate Induced Reticuloendothelial Blockade to Affect Plasma Levels of Factor VIII

By Evelyn Gaynor and Theodore H. Spaet

The site of antihemophilic globulin (Factor VIII) synthesis is unknown. Since implication of the reticuloendothelial system (RES) has been suggested by several investigators, and others have suspected a splenic role, the effects of RES blockade on plasma levels of Factor VIII have been studied.

Methyl palmitate induces selective depression of the RES, as measured by impaired phagocytic activity and depressed immune response. Unlike other RES-blockading agents, methyl palmitate causes neither liver parenchymal cell damage nor secondary hypertrophy of the RES cell mass. Moreover, its effect is relatively prolonged, causing impairment of colloidal carbon clearance for up to 72 hours after a single injection.

Methods and Materials

Sprague-Dawley male rats weighing 250–350 grams were used, and procedures were performed under ether anesthesia.

Palmitic acid methyl ester, obtained from Mann Research Laboratories, was prepared for intravenous injection according to a modification of the method described by Di Luzio and Wooles, in which the suspension was homogenized by ultrasonication for 25 seconds at 37 C. Animals were injected via the dorsal foot vein with 350 mg. of methyl palmitate suspension on 2 consecutive days, and Factor VIII assays and carbon clearance studies were performed 24 hours later. Control animals were given intravenous injections of the suspending medium alone.

Carbon clearances were studied essentially as described by Biozzi and associates, except that injections were given into the saphenous vein, and animals were given 5 mg. of heparin intravenously just prior to each experiment. Gunther Wagner Pelikan Ink, lot C 11/1431a, was injected in doses of 0.1 ml./100 grams. The optical density of the diluted hemolyzed samples were read in a Coleman Junior spectrophotometer at 650 mu with blood obtained just prior to carbon injection as the blank.

For coagulation studies, rat blood was collected by clean cardiac puncture into plastic syringes containing one-tenth volume of 3.8 per cent sodium citrate. Plasma was prepared by centrifugation for 10 minutes in a Clay Adams Sefuge and was assayed immediately.

Factor VIII activity was estimated as described by Hardisty and Macpherson, with the following minor modification: Crude brain cephalin, prepared according to Bell and Alton and diluted with buffered saline to give optimal activity in the test, was used instead of Inositol. All dilutions were made in isotonic saline brought to pH 7.2 with 1/10th volume of imidazole buffer (IBS). With this assay, the mean clotting times for 31 different rats was 41 seconds with a standard deviation of 2.8 seconds. A standard curve for Factor VIII was supported by grant HE-05414-05 from the National Heart Institute, USPHS, Bethesda, Md.

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activity was made from serial dilutions of pooled, normal rat plasma. The 10 per cent dilution was defined as having 100 per cent factor VIII activity. Twenty-four of 31 individual normal rat plasmas showed 70 to 200 per cent factor VIII activity.

RESULTS

The results of these experiments are shown in Figure 1. In animals treated with methyl palmitate, a mean carbon clearance $t^{1/2}$ of 36 minutes was obtained. Control animals treated with the suspending medium alone had a mean $t^{1/2}$ of 10 minutes. The phagocytic index $K$ was determined from the slope of the plot of log concentration of carbon against time, using the formula $\frac{dC}{dt} = KC$, where $C$ is carbon concentration. It can be seen that the mean $K$ for the methyl palmitate treated animals was 0.020 as compared with 0.068 for the control group. The mean Factor VIII levels of 140 per cent for the experimental group and 130 per cent for the control group were essentially identical, well within the range of normal values.

Three rats were given intravenous methyl palmitate daily, except for weekends, over a period of 2 weeks in doses totaling from 2.8 to 2.9 Gm. Factor VIII levels assayed 24 hours after the last injection were identical with baseline levels in two of the rats. Carbon clearance studies done on the third rat showed a $t^{1/2}$ of 50 minutes, with a phagocytic index of 0.012.

DISCUSSION

The effect of methyl palmitate on the RES appears to be selective, but it is not yet known whether synthetic function is inhibited along with phagocytic
function. Impaired phagocytic activity of the RES by both short-term and prolonged treatment with methyl palmitate did not affect plasma levels of Factor VIII in the rat. These studies indicate that either the RES does not make Factor VIII, that a population of RES cells escaped the methyl palmitate effect, or that Factor VIII synthesis by the RES is unrelated to its phagocytic activity. Unfortunately, a method for total RES ablation in mammals has yet to be devised.

**Summario in Interlingua**

Le effecto de palmitato methylic super le sistema reticuloendothelial pare esser selective, sed il es non ancora cognoscite si, a parte le function phagocytic de illo, etiam su function synthetic es inhibite. Le reducete activitate phagocytic del sistema reticuloendothelial inducite in le ratto per le application acute o chronic de palmitato methylic non se monstrava associate con alterate nivellos plasmatic de Factor VIII. Iste studios indica (1) que le sistema reticuloendothelial non produce Factor VIII o (2) que un subpopulacion de cellulas del sistema reticuloendothelial escapava al efecto de palmitato methylic o (3) que le synthese de Factor VIII per le sistema reticuloendothelial non es relationate con su activitate phagocytic. Infelicemente, un metodo pro le ablation total del sistema reticuloendothelial in mammiferos ha non ancora essite disveloppate.

**REFERENCES**

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