Lipid Mobilization by a Crystalline Peptide Isolated from Plasma of Horses Administered Cortisone.*† (23254)

JOSEPH SEIFTER AND DAVID H. BAEDER
Wyeth Institute for Medical Research, Radnor, Pa.

It has been reported that administration of cortisone resulted in the appearance in the plasma of an agent that inhibits the delactescing action of plasma obtained after heparin administration(1). This observation has been confirmed(2) and a less direct demonstration of this phenomenon has also been reported(3). In addition, plasma and its dialyzate prepared from plasma of horses administered cortisone (LM) caused hyperlipemia when administered to other animals(4,5) and patients(6). This effect occurred in the absence of adrenals or pituitary and differed from other hyperlipemia inducing agents such as protamine, pyrogens and DFP which required the presence of these endocrines(5). In this paper we are reporting data on the potency of crystalline LM.

Materials and methods. The sterile, pyrogen-free dialyzate was prepared as previously described by us(4). Preliminary chemical studies suggested that the active material was a polypeptide. The isolation and purification was in conjunction with our chemistry department and the details will be published elsewhere. Throughout the purification the samples were coded by the chemist and biological assays were run as unknowns. None of the operators analyzed any plasma sample for all lipids. Determinations of potency as a guide for purity were carried out in 865 male and female Wistar strain rats weighing 125-150 g. All animals and patients used in the present study were permitted only water for the 18-24 hours preceding injection of LM. The methods for determining plasma lipid levels were as previously described(5,6). The dose response curve with the final crystalline material was determined by injecting 10 intact rats at each of 10 logarithmically spaced doses. The minimal effective intravenous (recurrent saphenous vein) dose necessary to produce a 2-fold elevation in plasma cholesterol in 100% of the rats was 0.02 μg/kg. This dose was then similarly administered to 120 intact rats which were sacrificed in groups of 20 after 1, 2, 4, 6, 12 and 24 hours in order to determine duration of action. In addition, the same dose was injected intravenously into 20 hypophysectomized and 20 adrenalectomized rats, 60 guinea pigs, 6 dogs, 60 intact mice (tail vein), 12 intact rabbits (marginal ear vein) and into 3 patients at Temple University Hospital. Blood samples from the rats, guinea pigs and mice were obtained from the beating heart exposed under light ether anesthesia. All animals and patients were maintained in the fasted state until all blood samples had been obtained. The animals used in this and our previous studies had been subjected inadvertently to one of the following treatments: (1) The dogs were dipped in a chlordan bath; (2) all other animals were periodically sprayed with an insecticide containing piperonyl butoxide; (3) rabbits and guinea pigs received a supplementation of 10 g of chlortetracycline/ton of chow from the time of weaning; (4) similarly, rats and mice received a supplementation of 4 g of procaine penicillin/ton of food which contained in addition 0.005% butylated hydroxyanisole.

Results. Fig. 1 shows dose response relationship between crystalline LM and cholesterol concentration in the plasma. The cholesterol was determined for each rat. It is of interest that rats could respond with an

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TABLE I. Effect of Crystalline LM 0.02 μg/kg on Plasma Lipids (mg %) of Various Species.

Species	Hr after injection																	
	0			1			2			4			6			12			24		
	CH	FA	LP	CH	FA	LP	CH	FA	LP	CH	FA	LP	CH	FA	LP	CH	FA	LP	CH	FA	LP
Rats, intact	64	100	5	188	214	9	302	350	10	392	353	10	360	408	12	212	280	9	180	220	8
" hypox	56	80	5				248	313	11												
" adrex	58	91	5				250	336	10												
Mice	52	90	5	194	238	10	266	408	14	309	364	15	358	412	14				68	102	6
Guinea pigs	85	104	5	185	200	9	250	272	10	335	350	11	390	414	12				82	91	6
Rabbits	64	92	5	196	250	10	248	320	11	332	390	13	374	418	14				76	140	8
Dogs	124	160	6	200	352	11	390	400	18	418	404	19	402	440	18				196	220	9
Humans	220	230	7	390	440	13	420	500	15	490	560	16									

CH = Cholesterol. FA = Total fatty acids. LP = Lipid P.

FIG. 1. Dose response of rats to IV crystalline LM (10 rats/dose).

8 to 9 fold elevation of plasma cholesterol shortly after injection with 2000 times the minimal effective dose. Doses larger than this produced no further elevation. Table I presents the values for the plasma concentrations of cholesterol, total fatty acids and lipid P at various times after LM administration into the species tested. The response is manifested after one hour; however, the duration of action of this dose was different for each species.

Discussion. The 10,000 animals used in this and in our previous studies had been exposed to conditions considered to be harmless but which when intensified have in each instance been reported to produce fatty liver (7-11). It would appear, therefore, that the hyperlipemic action of LM is most consistently obtained in animals thus sensitized by hepatotoxic agents such as insecticides, antibiotics and antioxidants. The average concentration of cholesterol and total fatty acids in liver from 10 of our sensitized rats after a 24 hour fast was 3.1 and 3.7% respectively as compared with an average concentration of 0.6 and 1.1% in liver from 10 rats which had been allowed to recover for 3 weeks from exposure to the hepatotoxic agents. The average concentration of both lipids (1.9 and 2.7%) in the liver of 10 sensitized non-fasted rats was also higher than the values reported for the Wistar strain(12). These data suggest that animals treated with potential hepa-

totoxic substances either respond with greater mobilization of lipids to the liver following injection of LM, or that hepatotoxic agents interfere with utilization of mobilized fat brought to the liver. Our rats responded to a 24 hour fast by depletion of liver glycogen similar to that reported for the Wistar strain (13). The fact that hyperlipemia appeared in all the species tested indicates that the action of LM is not species or strain specific. The potency of crystalline LM, its activity in hypophysectomized rats and the failure of hypophysectomized rats to produce LM suggest the hormonal nature of this material. The increased purity resulting from crystallization was due largely to the removal of inorganic solids from the dialyzate. Preliminary studies indicate that the active material is a peptide that has neither vasopressor nor oxytoxic actions.

Summary and conclusions. (1) The active hyperlipemia inducing constituent of the dialyzate obtained from the plasma of horses administered cortisone has been crystallized and tentatively identified as a peptide. (2) Injection of 0.02 μg of crystalline material/kg IV induced marked hyperlipemia in intact mice, rats, guinea pigs, rabbits, dogs and humans as well as in hypophysectomized or adrenalectomized rats. (3) The animals used in this study were exposed to potential hepatotoxic agents.

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Infectious Bovine Rhinotracheitis (IBR). I. Propagation of Virus in Cancer Cells of Human Origin (HeLa). (23255)

V. J. CABASSO, R. G. BROWN, AND H. R. COX

Viral and Rickettsial Section, Research Division, and Veterinary Professional Service Department, Farm and Home Division, Am. Cyanamid Co., Pearl River, N. Y.

Infectious bovine rhinotracheitis (IBR) virus was successfully grown in tissue cultures of bovine embryo kidney, testicle and lung by Madin and others(1), and carried by them through 37 serial transfers in kidney tissue. These authors, however obtained no indication of cytopathogenic action of the virus on HeLa, KB or L cells or chicken fibroblasts. The present communication reports propagation of IBR virus in HeLa cell cultures through 26 serial passages.

Materials and methods. Virus strain: The virus used in this study was obtained from Drs. J. A. Baker and J. H. Gillespie, Veterinary Virus Research Inst., Cornell University, Ithaca, N. Y., to whom we are grateful. It was received in this laboratory as a frozen lung specimen from an experimentally infected calf, sacrificed while showing symptoms of IBR. *Tissue cultures*: Trypsinized stationary tube cultures of bovine embryo kidney, lung and skin-muscle were prepared