in HeLa cell tissue cultures. This compound inhibited GD VII virus in our tissue culture system. The hemagglutination titer was reduced 75% by a concentration of 0.1 mg/ml.

Discussion. Uridine does not inhibit virus and partially reverses inhibition produced by some substituted uridine nucleosides(3). As shown above uridine-5'-phosphate also reverses the inhibitory effect of the nucleoside derivative. 5-hydroxyuridine. The greater activity of cytidine derivatives in comparison with uridine derivatives as shown here is similar to the findings for *Neurospora*(9). Although chlorpromazine was active, *in vitro*, it failed to influence the infection in mice. The quantity used in the tests was nearly the maximal amount tolerated since it was found in preliminary studies that 2 mg daily was a lethal dose. The dose of 1 mg used resulted in generalized muscular weakness from which recovery was gradual and not complete as long as 24 hours later. The drugs tested probably did not act by interfering directly with the hemagglutination test since control tests with representative drugs from each group and virus incubated for 1 hour at room temperature gave the same hemagglutination titer as virus alone with one partial exception; the RBC sediment pattern in the presence of 0.1 mg/ml quinine dihydrochloride did not have as definite an endpoint as the control tube with virus.

Summary. Certain pyrimidine-related-compounds inhibit Theiler's virus, *in vitro*. Inhibition by 5-hydroxyuridine is partially reversed by uridine-5'-phosphate. Of various other chemicals found to inhibit virus, *in vitro*, chlorpromazine and polymyxin did not protect mice from viral infection.

TABLE II. Reversal of 5-Hydroxyuridine Inhibition by Uridine-5'-phosphate.

	% reduction of hemagglutination titer
5-hydroxyuridine, .5 mg/ml	75
Uridine-5'-phosphate, .5 mg/ml	0
5-hydroxyuridine, .5 mg/ml + uridine-5'-phosphate, .5 mg/ml	50
5-hydroxyuridine, .5 mg/ml + uridine-5'-phosphate, 1 mg/ml	0

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Received July 13, 1956. P.S.E.B.M., 1956, v93.

Relation of Endocrines and Clearing Factor Inhibitors to Hyperlipemia in Fasted Animals.*† (22664)

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

A variety of substances inhibit the clearing action of lipoprotein lipase *in vitro*. These include protamine(1), polylysine(2), diisopropylfluorophosphate (DFP)(3), iodinated thyronines(4), Na cholate(5), and an inhibitor (LM) present in plasma of rats receiving cortisone(6). Of these protamine(7), polylysine (2), Na cholate(5), and LM(8) have been reported to produce hyperlipemia when injected

* Presented in part at Fed. Am. Soc. Exp. Biol., April 16-20, 1956, Atlantic City, N. J.

† We wish to acknowledge the technical assistance of Wm. H. Bechmann, J. Dachiw, J. R. Howitz, M. R. Klenk, E. E. Livingston, Jr. and R. Reifsnyder.

into intact animals, presumably by inactivating lipoprotein lipase *in vivo*. LM, apparently produced by the pituitary, induced hyperlipemia in adrenalectomized and hypophysectomized rats and may be considered to either activate the fat depots or to inhibit lipoprotein lipase. It was of interest therefore to establish whether other hyperlipemia inducing agents act similarly. The failure of these to produce hyperlipemia in the absence of the adrenals or pituitary would suggest that the effect in intact animals could not be due to direct inactivation of lipoprotein lipase *in vivo* but rather the result of mediation through release of LM.

Materials and methods. To demonstrate the effect of food intake on the hyperlipemia inducing action of LM, 6 groups of 5 male and 5 female Wistar strain rats weighing 125-150 g were used. Three groups were maintained on the laboratory basal diet of Purina Chow checkers containing 5% fat up to the time of injection (Category A) and 3 groups were fasted for 20 hours preceding injection (Category B). Neither category received food from the time of injection until bled. One group of each received 5 mg LM kg either IV, IP or IM. Blood samples were obtained at the end of 2 hours. For the other rat studies all animals were fasted 18-24 hours and all injections were made into the recurrent saphenous vein. Solutions of compounds to be tested were freshly prepared in physiological salt solution just prior to use and the concentration was adjusted so that 0.1 ml/100 g of body weight was administered. Blood was obtained by cardiac puncture through the open chest while the animal was under light ether anesthesia. At this time it was verified that the stomach of fasted rats contained no food. The blood was mixed with 0.1 M Na citrate (1:9) and centrifuged in a constant temperature room (5° ± 1°C) until separation of the plasma occurred. Total cholesterol was determined by the method of Schoenheimer-Sperry(9), total fatty acids by that of Stoddard and Drury(10), and lipid P by that of Fiske and SubbaRow(11). Adrenalectomized rats maintained on Purina Chow checkers and 0.9% NaCl in their drinking water, and hypophysectomized rats fed white bread, Pard dog food, and oranges were used 10-14 days after operation. Completeness of adrenalectomy and hypophysectomy was checked in each rat at autopsy. In another study 3 male and 3 female mongrel dogs previously fasted for 18 hours were injected intravenously as described below. The previous diet of these dogs contained approximately 5% fat. Lipid values were determined, as described for the rats, on blood samples obtained from the recurrent saphenous vein before, during, and after each experiment. At least 7 days were allowed between studies and blood samples were drawn during these intervals.

Protamine sulfate (Lilly) of fish origin was administered to 20 fasted intact rats, 20 fasted adrenalectomized rats, 20 fasted hypophysectomized rats and to the dogs in doses of 10 mg kg. Blood samples were obtained from rats 1 hour after the injection and from dogs at hourly intervals for 6 hours. DFP supplied by Drs. John W. Gofman and Bernard Shore was administered to dogs in doses of 0.05, 0.10, and 1 mg kg and to 7 fasted intact rats, 7 fasted adrenalectomized rats and 7 fasted hypophysectomized rats in a dose of 1 mg/kg. Blood samples were obtained from the dogs at hourly intervals for the first 6 hours after injection and at the 24th hour and from rats 2 hours after injection. Pseudomonas polysaccharide (Piromen, Travenol Laboratories) was administered to 20 fasted intact rats, 20 fasted adrenalectomized rats and 20 fasted hypophysectomized rats in a dose of 50 μg/kg. Blood samples were taken 2 hours after the injection. The lipid mobilizer (LM) was prepared from the plasma of cortisonized horses as previously described(8) and was administered to 20 fasted intact rats, 20 fasted adrenalectomized rats and 20 fasted hypophysectomized rats and to the dogs in 1 mg/kg doses. Blood samples from rats receiving LM were obtained 1 hour after injection and from dogs at hourly intervals for the first 6 hours and at the 24th hour.

Results. Table I shows that injections of LM produced hypercholesterolemia only

TABLE I. Effect of Food Intake on Hypercholesterolemia Produced by LM in Rats.

	Total plasma cholesterol (mg %)*		
	IV	IM	IP
Fasted	184.3 ± 3.5	148.6 ± 3.2	168.6 ± 3.6
Non-fasted	60.0 ± 2.6	61.8 ± 2.0	64.0 ± 1.8

* Avg 10 rats/group ± S.D.

when injected into fasted rats but not in those which had received food up to the time of injection. The hyperlipemia in fasted rats receiving LM is illustrated in Fig. 1 and the values for each lipid component represent the averages for 20 rats (S.D. ± 5%). The hyperlipemic action did not depend upon the presence of either adrenals or pituitary. Fig. 2 shows the degree of hyperlipemia in rats receiving protamine. Similar results were obtained with rats administered either DFP or piromen. As with LM, in intact rats they produced a 2-3 fold elevation of all classes of lipids and a marked increase in optical density. In contrast, however, neither protamine, DFP nor piromen produced hyperlipemia in the absence of either adrenals or pituitary. The rats administered the DFP had convulsions and those given piromen had elevated rectal temperatures of at least 2.5-3°C. In the dogs receiving LM there was a 2-3 fold increase in all lipid components in 90-180 minutes which persisted for at least 3 hours. The plasma lipid values were in the normal range 24 hours after injection. Protamine administered to these same dogs at a later date also produced an increase in plasma lipid values, but the effect was less intense, of shorter duration, and the dose required was 10 times greater than that of LM. Similar administration of DFP to these dogs also produced hyperlipemia resembling that produced by protamine, but this occurred only after convulsant doses. The hyperlipemia did not appear after the dogs were administered non-convulsant doses of DFP although they exhibited unequivocal signs of anticholinesterase effects.

Discussion. LM induced hyperlipemia only in animals that were primed for lipid mobilization by starvation. It should be pointed out that all animals used in our studies were maintained on a low fat diet. In previous investigations of lipid mobilization diet, degree of fasting, or pretreatment with glucose had marked influences on the response (12). Of the many substances that produce hyperlipemia, particular significance has been attached to protamine(7) because this compound is considered to be a specific antagonist of heparin. Therefore the hyperlipemia it induces has been attributed to inactivation of lipoprotein lipase and has been presented as

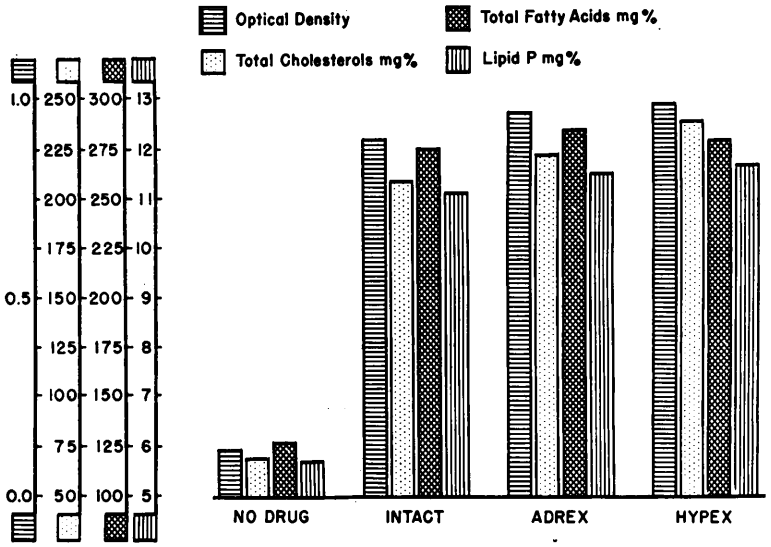

FIG. 1. Hyperlipemia by 1 mg LM/kg IV in rats (1 hr $\bar{p}$ inj.).

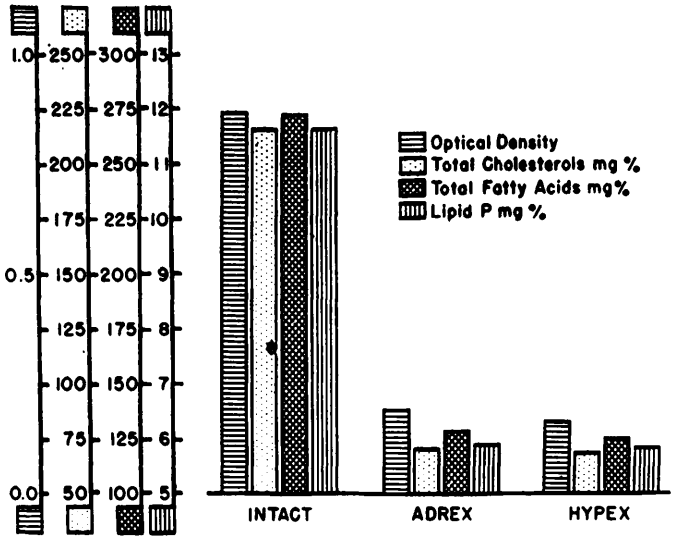

FIG. 2. Hyperlipemia by 10 mg protamine SO_4/kg IV in rats (1 hr $\bar{p}$ inj.).

evidence for the presence of heparin in the enzyme. However, the effective dose of protamine(7) is far in excess of that needed to neutralize the amounts of circulating heparin that can be detected by present methods and is within the toxic range that activates the pituitary and adrenals(13). Of particular significance is the large dose of protamine required to produce the hyperlipemia which was less intense than that produced by LM. Administration of larger doses resulted in more intense hyperlipemia which was accompanied by gross hematuria, prolonged shock with terminal asphyxial convulsions. The failure of protamine to produce hyperlipemia in adrenalectomized and hypophysectomized rats establishes that the action was not due to a direct effect on lipoprotein lipase but was mediated through the adrenal-pituitary system. Spitzer has also presented indirect evidence that protamine hyperlipemia involves more than anti-heparin activity(14). Shore has demonstrated that DFP is a potent inhibitor of lipoprotein lipase *in vitro*(3). Therefore administration of DFP in amounts to achieve similar concentrations in the plasma should result in hyperlipemia as a consequence of inactivation *in vivo*. We could observe no hyperlipemia in intact animals until convulsions had occurred which required administration of several times this amount. Furthermore, the hyperlipemia did not occur in adrenalectomized or hypophysectomized rats receiving the same doses and exhibiting convulsions. These results establish that the hyperlipemia due to DFP is a non-specific one mediated through the adrenals and pituitary and is not related to its anti-lipoprotein lipase activity. Since LM was obtained only from animals with intact pituitaries and induced hyperlipemia in the absence of either the adrenals or pituitary, it may be assumed to be released through the pituitary and to be the mediator through which stressors act as hyperlipemic agents. Administration of heparin releases lipoprotein lipase, but more direct evidence than hyperlipemia produced by protamine or DFP is needed to establish the presence of a heparin moiety in this enzyme or that it is involved in the physiologic or pathologic transport of lipids. The results with piromen establish that this agent also induced hyperlipemia by activating the pituitary and adrenals. Piromen has not been shown to inactivate lipoprotein lipase and therefore furnishes an excellent example of a non-specific stressor activating the pituitary-adrenal system to produce hyperlipemia.

Summary. 1. LM produced hyperlipemia only in animals primed for lipid mobilization. 2. Intravenous injection of toxic doses of pro-

tamine sulfate, convulsant doses of diisopropylfluorophosphate and pyrogenic doses of piromen produced hyperlipemia in intact animals. 3. The same doses of these substances injected into adrenalectomized and hypophysectomized rats did not produce hyperlipemia. 4. The hyperlipemic action of protamine or DFP is not attributable to direct inhibition of lipoprotein lipase but to a secondary effect mediated through the adrenals and pituitary. 5. The hyperlipemic action of protamine, DFP, pyrogens and possibly other stressors is best explained by release of LM.

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Received July 13, 1956. P.S.E.B.M., 1956, v93.

Evaluation of Germicidal Efficiencies of a Group of Antibiotics Tested by Tissue Culture Technic. (22665)

A. J. SALLE AND B. R. AMESUR.

Department of Bacteriology, University of California, Los Angeles.

An improved one-step tissue toxicity technic was proposed recently(1) for evaluation of germicides intended for clinical application.

The germicides were tested for their effect on living embryonic chick heart tissue fragments as well as for their ability to kill bacteria. A number known as the Toxicity Index was calculated from the results, which was defined as the ratio of highest dilution of germicide required to prevent growth of the tissue fragments in 10 minutes to highest dilution required to kill the test bacteria in the same period and under similar conditions. Theoretically an index less than one means that the germicide is more toxic to the test organism than to the embryonic tissue fragments; an index greater than one means that the germicide is more toxic to the tissue fragments than to the bacteria. The smaller the index the more nearly perfect the chemotherapeutic agent. There is no relation whatsoever between a phenol coefficient and a toxicity index.

The same procedure was followed for evaluation of the germicidal efficiencies of a group of antibiotics including: Bacitracin, Chloramphenicol (Chloromycetin) Penicillin G sodium, Polymyxin B sulphate, Streptomycin sulfate, and Terramycin HCl.

Aureomycin (Chlortetracycline HCl) and Achromycin (Tetracycline HCl) precipitated from saline shortly after being prepared and could not be evaluated. Likewise, Erythromycin failed to dissolve in saline.

Methods and materials. Embryonic chick heart tissue fragments from embryos 12 days old were used. Hearts were selected because of the ease by which a constant source of tissue could be obtained. The hearts were removed from the embryos and minced into fragments 0.5 to 1.0 mm in diameter by means of a sharp knife. The fragments were