ated the body temperature fall.

The exact age of the animals was not known but their size (225-275 g) indicates that they were well over 60 days of age with well developed temperature regulating mechanisms(7).

The quantitative data presented here support our hypothesis that struggling and thermolability (hypothermia) are positively correlated in the restrained rat. We have no ready explanation for this apparent paradox. However, these data do negate the contention of some that body temperature drop in the restrained rat is a result of muscular inactivity.

Summary. Body temperature drop is positively correlated with gross body movement of rats restrained in the cold. It is concluded that restraint hypothermia cannot be explained on the basis of restricted muscular activity.

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Independence of Phosphatide Induced Hypercholesteremia and Hepatic Function.* (22510)

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Previous studies(1-5) have suggested that the pathogenesis of various hypercholesteremias rested primarily on the retention and accumulation ("trapping") of cholesterol in plasma and plasma alone. Since phospholipid and/or neutral fat rises accompany all hypercholesteremic states, studies were done first with both these lipids together(6) and then with each lipid separately(7). The results showed that a prior plasma accumulation of either neutral fat or phospholipid was quickly succeeded by hypercholesteremia.

In the present study, the role of the liver in the hypercholesteremia induced by prior elevation of plasma phospholipid was investigated. This type of hypercholesteremia could be produced in the complete absence of functioning liver tissue.

I. *Cholesterol content of liver before and after induction of phosphatide induced hypercholesteremia.* To determine whether cholesterol accumulating in plasma following sustained hyperphospholipidemia(7) was accompanied by hepatic depletion of cholesterol, the following experiments were done.

Methods. Fifty male rats of Long Evans strain (Wt: appx. 315 g) between 14-18 weeks of age and which had been starved for 18 hours were used. Fifteen of these were injected intravenously with 3.0 ml of a 2% soybean phospholipide† solution containing 5% dextrose, and then injected continuously at an approximate rate of 1.0 ml per hour for 24 hours with the same concentration of phosphatide solution. Plasma samples obtained before and 24 hours after the beginning of

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† This phospholipide mixture was furnished by Glidden Co., labeled "Soya Lecithin, 'oil-free' RG Brand." Prior to injection, the soy phosphatides were freed from $Ca_3(PO_4)_2$. Phosphatides were further purified before injection into 10 of the 15 rats by successive reprecipitation from diethyl ether and from glacial acetic acid. The reprecipitated product showed no apparent sterol content by our analytical methods.

TABLE I. Cholesterol Content of Rat Plasma and Liver after Phosphatide Injection.

No. of rats	Avg wt (g)	Vol infused (ml)	Plasma total cholesterol (mg/100 ml) Before	Plasma total cholesterol (mg/100 ml) 24 hr after	Plasma phospholipid (mg/100 ml) Before	Plasma phospholipid (mg/100 ml) 24 hr after	Liver cholesterol Dry wt liver (g)	Liver cholesterol mg/100 g tissue	Liver cholesterol mg/organ
Control rats untreated									
15	307						*	859 ± 29†	29*
Control rats injected with dextrose solution									
20	318	23	59	66 ± 3.9†	113	118 ± 3.1†	2.86	891 ± 17	26 ± 0.8†
Rats injected with 2% phosphatide solution									
15	316	25	56	158 ± 12	110	408 ± 44	2.68	1200 ± 49	32 ± 0.8

* Analysis of single liver lobe only, except 5 animals where whole liver was used.
† ± S.E.

infusion were extracted with 1:1 alcohol:acetone and the extract analyzed for total cholesterol(8,9), and phospholipid(10). The liver was removed at 24 hours and cholesterol content determined as described previously for feces(11). Twenty control rats were treated in a manner identical to the above rats except that their infusion consisted of only 5% dextrose. The remaining 15 rats served as untreated controls.

Results. Continuous infusion of phosphatide solution into the 15 rats led to elevation of average plasma phospholipid content (see Table I) from 110 to 408 mg per 100 ml in 24 hours. The average plasma cholesterol also rose from 56 to 158 mg per 100 ml (over 180%) during this same period. Plasma phospholipid and cholesterol of control rats receiving dextrose solution (Table I) showed no significant change. The initial average cholesterol concentration of livers (as estimated by the analyses of the livers of the untreated rats) was approximately 859 mg per 100 g of liver, but after 24 hours of sustained hyperphospholipidemia, the average cholesterol concentration of the liver had increased to 1200 mg per 100 g. Infusion with dextrose alone did not change the liver cholesterol concentration significantly (Table I).

II. *Excretion of biliary cholesterol and cholate during phosphatide induced hypercholesteremia.* To determine whether such hepatic excess cholesterol concentration resulted from some intrinsic change in sterol metabolism within the liver, the excretion of bile cholesterol and cholate were measured.

Methods. The bile ducts of 6 rats were cannulated and bile collected while the animals were confined in a Bollman(12) type cage for 12 hours. During this same period 3 of the rats received 2% phosphatide solution continuously by vein. The remaining 3 rats served as controls receiving 5% dextrose solution. Plasma samples obtained at the beginning and at the end of infusion were analyzed for total cholesterol and phospholipid. The bile was analyzed for total cholesterol by extracting(13) or by direct extraction with alcohol:acetone, precipitation of the cholesterol as the digitonide, and analysis of the digitonide(9). It was analyzed for bile acid by absorption spectrophotometry(14).

Results. The induction of hypercholesteremia by elevation of plasma phospholipid did not appear to be accompanied by any significant change in the production of either bile cholesterol or cholate (Table II). The average biliary cholesterol excretion of hypercholesteremic rats was 1.6 mg in 12 hours as compared to 1.8 mg in control rats. Biliary excretion of cholate also appeared to be the same in both groups. This evidence suggests that the normal course and rate of cholesterol metabolism by the liver remains unchanged in agreement with previous findings(15,16).

III. *Effect of subtotal and total (functional) hepatectomy upon phosphatide induced hypercholesteremia.* The preceding data strongly suggested that excess of cholesterol found in plasma was not arising from liver but was itself effecting a rise in liver cholesterol. The following experiments therefore were done to explore this possibility.

Methods. A series of 20 rats were sub-

TABLE II. Effect of Phosphatide Injection on Bile Cholesterol and Cholate Content in Rats; 3 Rats in Each Series.

Wt (g)	mg phos. inj.	Plasma cholesterol Before (mg/100 ml)	Plasma cholesterol 12 hr (mg/100 ml)	Plasma phospholipid Before (mg/100 ml)	Plasma phospholipid 12 hr (mg/100 ml)	Bile vol (ml)	Bile cholesterol mg/100 ml	Bile cholesterol mg/12 hr	Bile cholate mg/100 ml	Bile cholate mg/12 hr
				Rats given phosphatides						
307	282	60	145	107	346	6.7	23	1.6	230	14
				Rats given dextrose						
309	None	63	73	123	167	7.7	27	1.8	192	14

jected to complete, and 8 rats to subtotal, hepatectomy by previously described methods (17). Immediately after hepatectomy, all rats were given intravenously 1.5 ml of 2% phosphatide solution in 5% dextrose and then infused with the same solution at an average rate of 1.0 ml per hour. Infusion was continued in totally hepatectomized rats for 6 hours and in partially hepatectomized rats for 12 hours. Plasma samples obtained before, and at the end of, infusion were analyzed for cholesterol and phospholipid. In some experiments, packed red blood cell volume was determined at both the beginning and the end of infusion. No significant change in hematocrit was observed. For control purposes, 15 normal rats were subjected to the same program.

Results. A rise in plasma cholesterol quickly occurs after infusion of soy phosphatides into the partially or totally hepatectomized animal (Table III). As a matter of fact, average plasma cholesterol content of the totally hepatectomized rat increased more (46 to 127 mg per 100 ml; an increase of approximately 176%) after 6 hours of infusion than that of the infused normal rat (56 to 96 mg per 100 ml; an increase of approximately 71%). Similarly average plasma cholesterol of the partially hepatectomized rats increased more (56 to 197 mg per 100 ml; an increase of approximately 250%) after 12 hours of infusion than that of the normal rat (56 to 131 mg per 100 ml; an increase of approximately 134%) after the same period of infusion.

Discussion. The above findings confirm our earlier studies(7) indicating that an hyperphospholipidemia induced by *continuous* infusion of mixed phosphatides sufficient to maintain a continuously high level of plasma phospholipid quickly leads to hypercholesteremia. Furthermore, the degree of hypercholesteremia is dependent seemingly not upon the amount of phosphatide infused but upon the excess amount persisting in plasma. Thus our hepatectomized animals received no more phosphatide than control animals but because of their relative inability to rid plasma of phospholipid administered, a greater accumulation of these substances occurred in plasma and this in turn presumably provoked a more severe hypercholesteremia. The above studies also indicate that the excess cholesterol present in hypercholesteremia

TABLE III. Plasma Cholesterol Content of Hepatectomized Rats after Phosphatide Injection.

No. of rats	Avg wt (g)	Avg % liver remaining	Total cholesterol (mg/100 ml) Before inj.	6 hr after inj.	12 hr after inj.	Total phospholipid (mg/100 ml) mg % lipid phosphorus × 25 Before inj.	6 hr after inj.	12 hr after inj.
				Control rats with intact livers				
15	306	100	56	96 ± 4.5*	131 ± 15*	103	206 ± 7.4*	219 ± 42*
				Rats with subtotal hepatectomy				
8	313	33	56		197 ± 20	87		420 ± 44
				Rats with complete hepatectomy				
20	305	None	46	127 ± 6.9		107	632 ± 43	

* ± S.E.

induced by injection of soy phosphatides is apparently derived primarily from extrahepatic sources. Furthermore, unlike other forms of hypercholesteremia (*e.g.*, nephrosis (3), biliary obstruction(1) and Triton induced hypercholesteremia(5)), the liver of the rat with phosphatide induced hypercholesteremia demonstrates an increased concentration of cholesterol. These findings are explainable if it is remembered that in phosphatide induced hypercholesteremia plasma phospholipid content is elevated by infusing phosphatide at a rate faster than the ability of liver to remove phospholipid. Removal of some phospholipid by liver takes place as usual(18). During this continual normal hepatic removal of phospholipid, probably some of the excess plasma cholesterol is also removed.

Since, according to the present studies, intravenously administered phosphatide is capable of mobilizing cholesterol from extrahepatic sources and depositing at least some of this cholesterol in the liver of the normal animal, the administration of phosphatides appears to have therapeutic implications. Of course the administration of phosphatide to an animal already exhibiting a deficiency in its phospholipid turnover and a consequent hyperphospholipidemia would only intensify both the latter and the hypercholesteremia it also had evoked(7). Phosphatide then may well be a double edged sort of physiological sword in that it evokes hypercholesteremia when its own egress from plasma is faulty but conversely can mobilize and bring extrahepatic cholesterol to the liver for disposal if its own egress is assured.

Summary. (1) If sufficient phosphatide is intravenously administered to the rat to induce a *continuous* state of hyperphospholipidemia, a state of hypercholesteremia also ensues. The hepatic concentration of cholesterol also is increased. (2) Phosphatide induced hypercholesteremia appears independent of sterol metabolism of liver as judged by assay of bile for cholesterol and cholate contents. Furthermore, total or partial hepatectomy increased the degree of hypercholesteremia induced by experimental hyperphospholipidemia. (3) Mobilization of extrahepatic cholesterol and its subsequent deposition in the liver by experimental induction of hyperphospholipidemia appears to have possible therapeutic implications.

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