duration of the acetylcholine effects indicates the complexity of the reaction since the acetylcholine must be rapidly split by the esterase in the cortical tissue.

Summary. 1. Topical application of acetylcholine usually produced a local depression. The depression was not propagated, and was not due to changes of systemic blood pressure. 2. The local depression was often succeeded and sometimes masked by enhanced electrical reactivity. This action varied from a barely discernible increase to a sustained high voltage pattern lasting 30 minutes. The discharges were randomly distributed in time in some instances; in others, the activity was organized in distinct groups of paroxysmal spikes each group being accompanied by a distinct surface negative D. C. pulse.

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Relationship of Native Heparin to Clearing of an Alimentary Lipemia.* (20177)

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The dramatic *in vivo* clearing by commercial heparin and heparin-like substances of an alimentary lipemia has been noted by a number of workers(1-5). This clearing has been shown to be reversible in dogs and rats by injections of protamine(6), a substance which is known to combine with and inactivate heparin(7). In the intact dog it has also been shown that the clearing produced by small amounts of intravenous heparin is not initiated in the circulation of the brain, but is initiated in the circulation of the leg and lung; once circulation is complete and mixing of the blood has occurred clearing continues in samples of blood from all of these sources(8).

To test the effects of native heparin on an alimentary lipemia, anaphylactic shock and shock following the injection of certain histamine liberators have been studied. In other experiments the effects of intravenous injections of dextran have been observed. These studies will now be reported.

Material and methods.† Dogs were sensitized with 50% eggwhite (1 ml/kg). The initial injection was given intravenously and 5 and 7 days later this dose was repeated subcutaneously. Thirty days later the dogs were fed cream fat (2-4 g/kg body weight), and 3-4 hours later anaesthesia with nembutal was induced. A control lipemic sample of blood was then drawn. The small shock dose of antigen was injected intravenously. Four

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TABLE I. Effects of Circulatory Shock, Protamine and Dextran on Blood Heparin and Alimentary Lipemia in Dogs.

Procedure	Dose range	No. of exps.	Response: Blood heparin*	Response: Visible lipemia
Anaphylactic shock	Shock dose: 1–5 cc 50% eggwhite	2	I	D
		1	I	N
		2	I	I
		1	N	N
		2	N	I
Compound 48/80	0.4–1.0 mg/kg	1	I	D
		2	I	I
		3	N	D
		1	N	I
DA 10	0.6 and 1.0 mg/kg	1	I	D
		1	I	V
Protamine (following shock)	2–4 mg/kg	3	D	I
		3	N	I
		1	N	N
Protamine†	8 mg/kg	1	I	I
Histamine	5 and 10 μg/kg	2	N	N
Dextran		3	N	N
		3	N	S
		3	N	D

* Changes in blood heparin activity refer to changes from normal values except in the case of "protamine (following shock)" where a decrease from elevated values (due to the preceding shock) occurred in 3 exp.

† Protamine sufficient to produce shock was inj. in this exp.

I = increase; N = no change; D = decrease; V = variable; S = slow decrease.

blood samples were then obtained during the first 10 minutes, and 2 more in the subsequent 20 to 30 minutes. In a few instances the animals died in approximately 10 minutes. The blood samples were drawn from the femoral artery into syringes containing 1/10 volume of 0.1 M sodium citrate. Sufficient blood was also drawn into heparin for determination of the chylomicron counts. Blood pressure was recorded from the carotid artery in order to estimate the severity of the anaphylactic shock. Experiments were also performed on normal cream-fed dogs which received small shocking intravenous doses of the potent histamine liberators diamino decane (DA 10) and compound 48/80,‡ blood samples being drawn as indicated above. In these the blood pressure usually fell to 40 to 70 mm of mercury within ½ minute after the injection. In other experiments the effect of protamine sulfate on fat clearing due to shock was investigated. Finally the effects of dextran§ (molecular wt 75,000) upon a cream lipemia were observed. In most cases fat clearing was estimated by inspection for visible turbidity of the plasma and by whole blood chylomicron counts under dark field illumination. Blood heparin was determined by metachromatic assay, after precipitation of the plasma proteins with 80% phenol(9). Antithrombic potency of the extracts(10) and thrombin inactivating power of the serum(11) were also estimated in many experiments. Clotting times were performed in duplicate by the capillary tube method.

Results. The results of our experiments are summarized in Table I. In 4 experiments the release of native heparin during anaphylactic shock or shock due to injections of histamine liberators was accompanied by clearing of an alimentary lipemia. On other occasions, however, we observed that the presence of large amounts of heparin in the blood during anaphylactic shock (3 experiments) and after injections of 48/80 (2 experiments) was not accompanied by lipemia clearing. In one experiment both whole blood chylomicron counts and the plasma turbidity were still unchanged

‡ Compound 48/80 was kindly supplied by Dr. E. J. deBeer of Wellcome Research Laboratories, Tuckahoe, N. Y.

§ Furnished by Commercial Solvents Corp., Terre Haute, Ind.

from the lipemic levels 14 minutes after the injection of the shocking dose of eggwhite, despite the presence of 1.55 metachromatic units (Mu) of native heparin per ml of blood. In 4 other experiments the lipemia (turbidity and chylomicron counts) actually increased following the onset of shock although the blood heparin continued to rise during the same period. (In our experience the normal range of blood heparin activity in dogs is 0.10 ± 0.08 Mu/ml and the threshold for lipemia clearing by commercial heparin may be less than 0.01 Mu/ml above the control value)(8).

Total clearing of a heavy lipemia may occur in dogs in the complete absence of antithrombic activity, and with no detectable increases in the level of metachromatic activity in the blood extracts. Also no increase could be detected in the thrombin inactivating power of the serum. In 3 experiments this was observed after the intravenous injection of 8 cc of 15% dextran (M.W. 75,000) into lipemic dogs, in which moderate to marked clearing occurred within a few minutes.‖ It is to be noted that these animals received several injections of dextran at intervals of about 1 week, making it possible that they had become sensitized to the dextran. This could have been a factor only in one experiment in which the maximum clearing was noted. Clearing of the lipemia in the absence of a detectable increase in native blood heparin activity has also been observed after shock due to injections of compound 48/80.

Following the injection of protamine sulfate the lipemia which disappeared in the presence of heparin reappeared promptly in all of our experiments. Protamine sulfate also reversed the clearing effect of compound 48/80 in lipemic dogs, even when the clearing was accompanied by no demonstrable increase in heparin activity in the blood of the animals.

Discussion. The observation that the release of native heparin in the blood stream of dogs may be accompanied by rapid clearing of a heavy alimentary lipemia supports the view that heparin may play an important physiological role in fat transport and metabolism(8). However, in only 4 out of our 10 shock experiments involving a rise in blood heparin was there any finding to suggest that native heparin might be the primary cause of lipemia clearing. In 5 other experiments increases in blood heparin coincided with either no change or an actual rise in the visible lipemia of cream-fed dogs.

While injections of commercial heparin in minute amounts usually produce some clearing of a lipemia, we have observed that the presence of even *much greater* amounts of native heparin in the blood may not be accompanied by clearing. It is possible that the clearing may be inhibited by other substances released into the blood stream during shock, or as previously suggested(8) the mechanism of clearing may be exhausted by its too frequent or prolonged use. On the other hand in 6 experiments, marked clearing was observed in the absence of measurable increases in the levels of heparin in the blood.

Our observations do not support the statement that heparin is essential for the production of a lipemia clearing factor(12). Neither do they show that the presence of adequate amounts of heparin necessarily cause clearing. We have shown that the injection of extremely small amounts of heparin in dogs may result in transient slight lipemia clearing even when these doses are too small to become manifest as an increase in blood heparin concentration (8), an observation which suggests the possibility that the methods of heparin assay may not accurately disclose the effective amounts of heparin in the blood. However, the notable persistence of a lipemia in the presence of adequate amounts of native heparin and the very marked clearing sometimes observed in the absence of detectable amounts of heparin are difficult to explain. Possibly alternate pathways exist, independently of heparin, for lipemia clearing to occur.

Summary. Studies have been made to determine the effects of native heparin upon the lipemia of cream fed dogs. It was found that shock due to anaphylaxis or to the injection

‖ It has also been shown by one of us (R.L.S.) that dextran (N.W. 75000 and 210000) will clear a lipemia *in vitro* when added to heparinized blood in concentration of 1% and 2%. When added to lipemic plasma this clearing is either entirely absent or very much less marked.

of certain histamine liberators sometimes causes heparin to appear in the blood of the animals. The presence of native heparin in the blood, however, does not necessarily coincide with clearing of a lipemia; fat clearing may occur during shock either in the presence or in the absence of detectable amounts of native heparin in the blood of dogs. In several experiments, the injection of dextran (M.W. 75,000) produced marked clearing of a heavy alimentary lipemia in the absence of heparin activity in the blood of dogs. Protamine sulfate caused the return of both visible turbidity of the plasma and of whole blood chylomicron counts to control levels after fat clearing due to shock. This occurred both in the presence and absence of significant blood heparin activity.

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Comparative Study of Epinephrine and an Epinephrine-like Substance Obtained from Autolyzing Adrenal Glands.* (20178)

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During an assay for adrenalin from minced, autolyzing adrenal glands which have been placed in dialyzing bags it was noticed that the dialysate had acquired a bluish-violet color. The pressor substance could not be precipitated from the dialysate by the addition of strong ammonium hydroxide but yielded epinephrine on acid hydrolysis. When the glands were sliced with stainless steel knives the bluish-violet color was not obtained. Suspecting contamination from the food chopper, we added various metallic salts to a purified adrenalin solution and it was found that ferric ammonium oxalate gave a comparable color. Solvents such as ether, chloroform, benzene and carbon tetrachloride failed to extract the color. In comparing the bluish-violet dialysates with Parke Davis (P.D.)† adrenalin to which ferric ammonium oxalate had been added, 10% HCl was added to both solutions; the dialysate turned colorless and the solution of adrenalin and ferric ammonium oxalate became a light yellow color. When 10% NaOH was added to the adrenalin and ferric ammonium oxalate an orange precipitate formed, but when 10% NaOH was added to the dialysate it turned pink. Both solutions contained 0.05 mg of adrenalin per ml as shown by bio-assay. P.D. adrenalin was used as the standard.

Folin(1) has shown that uric acid and Doty (2) has shown that ascorbic acid, both contained in adrenal glands, can interfere with the formation of colored compounds of epinephrine and certain metallic salts. It was found that ascorbic acid prevents the formation of the bluish-violet color of ferric ammonium oxalate and adrenalin but that uric acid neither prevents the formation of the

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† Parke Davis adrenalin.