TABLE III.* Comparison of Complement-Fixing and Agglutinating Activity of 481 Human Sera to the LMcN SBE Antigen and Brucella Antigen Respectively.

C.F. titer to LMcN SBE	Brucella agglutination titers: Neg	20	40	80	160	320	640	1280
<8	355	18	21	9	7	3	4	1
8	13	0	0	1	1	0	0	0
16	6	0	0	0	2	0	1	0
32	18	1	1	0	0	0	0	0
64	9	0	2	1	0	0	0	0
128	7	0	0	0	0	0	0	0

* Numbers in table refer to numbers of individuals. One serum reported here is not reported in Table II.

Table I had C-F antibodies in their sera which were specific to the soluble antigen prepared from McNutt's strain of SBE virus. Furthermore, it would seem that the soluble antigen of the McNutt strain of the virus of SBE is distinct from the above described antigens prepared from members of the psittacosis lymphogranuloma venereum group of viruses. If the comparison of the titers of these 7 sera to the 9 antigens may be considered indicative of the specificity of the LMcN SBE antigen and the results tabulated in Tables II and III are accepted as further substantiation of this specificity, then it is evident that out of 488 human sera tested, 75 (15%) had a titer of 1:8 or higher and 51 of the 75 had a titer of 1:32 or higher to the virus of sporadic bovine encephalomyelitis. Further studies are in progress to determine the significance of these data.

Summary. The presence of complement-fixing antibodies to the virus of sporadic bovine encephalomyelitis has been demonstrated in 15% of the 488 human sera tested, with 51 of these 75 sera showing relatively high titers. The apparent specificity of the C-F antibodies to the virus of SBE has been demonstrated. The suggestion is made that SBE is yet another disease of animals whose causative agent is transmissible to man.

1. McNutt, S. H., *Vet. Med.*, 1940, v35, 228.
2. Menges, R. W., Harshfield, G. S., and Wenner, H. A., *J.A.V.M.A.*, 1953, v122, 294.
3. Wenner, H. A., Harshfield, G. S. Chang, Te Wen., Menges, R. W., *Am. J. Hygiene*, 1953, v57, 15.

Received January 25, 1954. P.S.E.B.M., 1954, v85.

Production of Lipemia Clearing Factor During Anaphylactoid Shock. (20921)

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It has been known for many years that, in dogs, the intravenous injection of peptone produces profound shock associated with incoagulable blood. Later it was shown that the clotting defect is due to the presence of heparin and that the mast cells of the liver of shocked animals are degranulated(1). Shortly thereafter it was demonstrated that protamine reverses the coagulation defect resulting from anaphylactic shock in dogs(2) and subsequently heparin was isolated from the blood of such animals(3). The source of the heparin is apparently the liver, for no heparin could be found in the blood of shocked, hepatectomized dogs(2). Recently many organic bases, termed "histamine liberators," have been found to produce shock with some of the characteristics of anaphylaxis (4). This also has been accompanied by the release of a heparin-like substance into the blood of dogs. In other mammals it has usually not been possible to demonstrate the presence of heparin in the blood during shock produced by these methods(5). Mast cell degranulation and disruption, however, has been found in rats following the administration of histamine liberators(6). It is well established that in many mammals the intra-

TABLE I. Summary of Data Obtained during Administration of Agents Used to Produce Anaphylactoid Shock in Dogs.

Agent used	No. of exp.	Shock produced in	Clotting time prolonged in	Anti-heparin agent given in	Incidence of significant clearing factor activity			
					Before shock	After shock	After anti-heparin agent 1 min.	10 min.
Peptone	7*	7	7	4	0	7	2	0
Compound 48/80	4	3	2	3	0	3	0	0
Stilbamidine diisoethionate	2	2	0	0	0	2	—	—
Total	13	12	9	7	0	12	2	0

* No anesthesia or diphenhydramine in 2 exp.

venous injection of heparin is followed by the appearance in the plasma of a lipemia "clearing factor" (CF) which catalyzes the lipolysis of chylomicrons and low-density lipoproteins(7). Because of the heparinemia existing in dogs in anaphylactoid shock, it seems likely that CF might also be present in their plasma. Levy and Swank(8) were occasionally able to demonstrate the clearing of alimentary lipemic plasma *in vivo* in dogs during anaphylactic shock or shock produced by histamine liberators, but they were unable to find any correlation between the occurrence of clearing and the presence of detectable blood heparin. They suggested that heparin is neither essential for, nor necessarily causes, the production of CF. However, they also found that protamine sulfate administration reversed the clearing of lipemia whether or not heparin could be detected.

In the studies reported below CF was invariably present in the plasma during anaphylactoid shock in dogs, indicating that endogenous heparin produces this substance as predictably as injected heparin or heparinoids.

Materials and methods. Peptone, Witte or Difco (0.2-0.3 g/kg), and the histamine liberators, stilbamidine diisoethionate (15 mg/kg) and a condensation product of p-methoxyphenyl-ethyl-methylamine and formaldehyde known as compound 48/80* (0.5-2 mg/kg), were used to produce shock. Mongrel dogs were anesthetized with intravenous sodium pentobarbital (28 mg/kg) and a slow saline infusion was started in a femoral vein. The total volume infused during the procedure was under 100 ml. Blood samples were taken and experimental solutions were injected through a 3-way stopcock. An aliquot of each sample to be analyzed for CF activity was mixed with 1/10 volume 1% ethylenediamine tetra-acetic acid in .15 M saline and chilled immediately in ice water(9). Clotting times were determined on 2 ml aliquots. A control blood sample was taken, following which 25 mg diphenhydramine hydrochloride was injected. This anti-histaminic compound was given to mitigate the severity and prevent a possible fatal outcome of the shock. Ten minutes after the first, a second control sample was taken and the shock-producing agent administered. Further blood samples were withdrawn at intervals up to one hour following induction of shock. In some experiments, an anti-heparin agent was injected immediately following the last sample and 2 additional samples taken one and 10 minutes later. The anti-heparin agent used was protamine sulfate (1-3 mg/kg) except in one experiment where 1,5-dimethyl-1,5-diazoundecamethylene polymethobromide† was used. Some dogs were used on more than one occasion, but several days were allowed to elapse between successive experiments on the same animal. Peptone was also administered to rats and guinea pigs. Only single blood samples were taken following the shock-producing injection in these animals. Clotting times were determined by a 2-tube Lee-White method. Increases of double the mean of the control values were considered significant.

* Kindly supplied by Dr. E. J. deBeers, Wellcome Research Laboratories, Tuckahoe, N. Y.

† "Polybrene," obtained through courtesy of Dr. G. H. Berryman, Abbott Laboratories, Chicago, Ill.

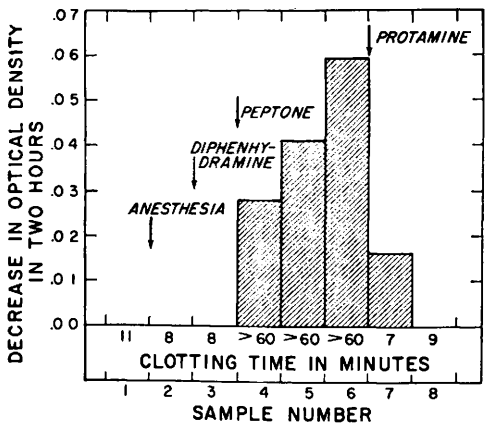

FIG. 1. Clearing factor activity during peptone shock. 13.6 kg mongrel dog. Sample numbers: 1, before sodium pentobarbital; 2, before diphenhydramine; 3, before peptone; 4, 5, 6, 10, 20 and 60 min. after peptone (0.2 g/kg); 7, 8, 1 and 10 min. after protamine sulfate (3 mg/kg).

CF analyses were performed by incubating one part of plasma with 9 parts of warmed substrate at 38°C and following the change in optical density of the mixture in an Evelyn photoelectric colorimeter at 515 mu. The substrate was prepared by adding to .15 M saline an amount of the chylous layer of lipemic human donor plasma sufficient to produce an optical density of 0.3-0.5. CF activity was considered significant when the optical density decreased at least .02 units in 2 hours.

Results. The results obtained in dogs are summarized in Table I. A representative experiment is shown in Fig. 1. It will be noted that significant CF activity was present every time that shock was produced, whereas it was never present prior to administration of the anaphylactoid agent.‡ On 3 occasions this occurred in the absence of an increase in clotting time. On the other hand, an increase in clotting time was never observed in the absence of CF activity. In one dog receiving 0.5 mg/kg of compound 48/80 there was no evidence of shock; in this animal only was no CF detected. The other animals given this drug received 1-2 mg/kg.

‡ Administration of diphenhydramine alone to 2 anesthetized dogs did not result in the appearance of significant CF activity up to 2 hours following injection.

On 6 occasions protamine sulfate was given one hour after induction of shock; on 5 of these CF activity was abolished in the samples taken one and 10 minutes later. In the sixth instance, CF activity was undiminished one minute after protamine in spite of a reduction of clotting time to normal, but was absent in the 10-minute sample. One dog received 2 mg/kg Polybrene instead of protamine. This substance caused a return of the clotting time to normal in one minute, while CF activity was still present, though markedly diminished. In the 10-minute sample CF activity was absent.

Shocked dogs usually became pulseless in 1-2 minutes. Recovery from shock usually began in 10-20 minutes and was complete in about an hour. CF activity was usually maximal 30 minutes or more after induction of shock.

The quantities of CF produced during anaphylactoid shock were often substantially less than those observed following intravenous heparin injections which produced similar increases in clotting time. It was postulated that this low activity might be a function of the shock state itself, since previous experiments done in dogs during traumatic or hemorrhagic shock had shown that very little CF was produced following heparin injection(10). To test this hypothesis a dog was given 126 U/kg heparin 2 minutes after a shock-inducing injection of peptone. Four days later the same dog was given this dose of heparin alone. Samples were taken for determination of CF activity just prior to heparin and 2, 5, 10, and 25 minutes following injection in both instances. Analyses were performed with the same substrate under identical conditions. The results are shown in Fig. 2. It is apparent that in the first case there was little increment in the CF activity already present until the circulation was partially restored. Further, the activity produced by the same amount of heparin in the absence of shock was several fold greater than that produced during shock. This experiment was repeated in another dog with peptone administration on the second occasion rather than the first. Again CF activity following heparin was much less in the shocked state.

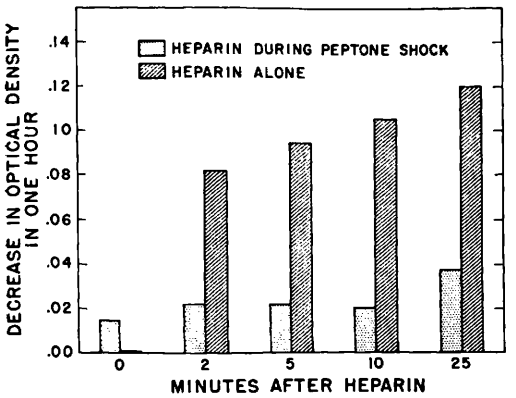

FIG. 2. Effect of peptone shock on heparin-induced plasma clearing factor activity. 12.3 kg mongrel dog. Heparin (126 U/kg) given 2 min. following 0.3 g/kg peptone.

The identity of the CF produced during anaphylactoid shock with that produced by heparin injection was confirmed in several ways. The activity fell in fraction III-1,2,3 by low-temperature alcohol fractionation(7) and required albumin as a fatty acid acceptor (11).§ In addition, as determined by ultracentrifugal analysis, plasma of a shocked dog caused a reduction in the concentration of low-density lipoproteins and an increased concentration of molecules with the flotation rate of alpha$_1$ lipoprotein when incubated with lipemic human serum(12).

Administration of shock-producing doses of peptone to rats resulted in no increase in clotting time nor in the appearance of significant quantities of CF. In guinea pigs, the injection of 0.4 g/kg peptone frequently was followed by the appearance in the plasma of slight, but significant CF activity which could be promptly abolished by protamine injection. No change in clotting time was ever observed in this species.

Discussion. Our finding that CF is invariably present during anaphylactoid shock in dogs contrasts with that of Levy and Swank(8) who could observe no constant correlation between the appearance of clearing activity *in vivo* and evidence of heparinemia. Methodological differences probably underly this discrepancy. The relatively small amounts of CF usually present during shock might not be detectable in the plasma of an animal still discharging lipid-laden thoracic duct chyle into the general circulation. Furthermore, under these conditions the effects of shock on intestinal absorption and lymphatic flow are uncontrolled. In the *in vitro* assay system the only variable factor is the ability of the plasma to decrease the optical density of the substrate.

The demonstration of CF activity in the absence of an increase in whole blood clotting time is not surprising, since it is well established that CF can be produced readily by amounts of heparin insufficient to alter clotting time(13), a relatively insensitive index of blood heparin concentration. However, Levy and Swank found evidence of clearing activity in dogs during shock in the absence of detectable blood heparin by the more sensitive methods of metachromatic assay and antithrombin activity of blood extracts, or thrombin inactivating power of the serum. Similarly, Adams(5) found no prolongation of clotting time in guinea pigs during anaphylactic or peptone shock. Available evidence would support the hypothesis that smaller amounts of heparin are required to produce detectable CF activity than to produce detectable alterations of the clotting mechanism. That this is true insofar as metachromatic assay of heparin is concerned has been shown previously(13).

It is clear that less CF is produced by heparin during peptone shock than in the normal animal. It appears likely that this is related to interference with the normal processes by which the CF precursor in plasma and/or heparin are made available to the tissue factor known to be essential for CF production(7). Other factors, such as CF inhibitors, cannot be ruled out, however.

The production of CF by endogenous heparin is consistent with other evidence obtained in this laboratory that heparin plays a physiologic role in lipid metabolism(14). The small quantities of heparin required for CF production relative to those required to affect blood coagulation combine to make this an attractive hypothesis. It has not yet been possible, however, to detect unquestionable

§ We are indebted to Dr. Robert Gordon for these determinations.

CF activity in normal blood by the methods now available.

Summary. Endogenous heparinemia produced during anaphylactoid shock in dogs is regularly accompanied by the appearance in the plasma of lipemia clearing factor identical to that produced by heparin injection. The significance of this finding is discussed in relation to the postulated role of heparin in lipid metabolism.

1. Wilander, O., *Skand. Arch. f. Physiol.*, 1938, v81, suppl. xv.

2. Waters, E. T., Markowitz, J., and Jaques, L. B., *Science*, 1938, v87, 582.

3. Jaques, L. B., and Waters, E. T., *Am. J. Physiol.*, 1940, v129, 389.

4. MacIntosh, F. C., and Paton, W. D. M., *J. Physiol.*, 1949, v109, 190.

5. Adams, S. S., *J. Pharm. and Pharmacol.*, 1953, v9, 580.

6. Riley, J. F., *J. Path. Bact.*, 1953, v65, 471.

7. Anfinsen, C. B., Boyle, E., and Brown, R. K., *Science*, 1952, v115, 583.

8. Levy, S. W., and Swank, R. L., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 553.

9. Brown, R. K., Boyle, E., and Anfinsen, C. B., *J. Biol. Chem.*, 1953, v204, 423.

10. Boyle, E., Unpublished observations.

11. Gordon, R. S., Boyle, E., Brown, R. K., Cherkes, A., and Anfinsen, C. B., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 168.

12. Boyle, E., Bragdon, J. H., and Brown, R. K., *ibid.*, 1952, v81, 475.

13. Swank, R. L., and Levy, S. W., *Am. J. Physiol.*, 1952, v171, 208.

14. Bragdon, J. H., and Havel, R. J., *ibid.*, in press.

Received January 27, 1954. P.S.E.B.M., 1954, v85.

Locus of Emetic Action Following Irradiation.* (20922)

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The central emetic mechanism has recently been resolved into 2 anatomically close but functionally distinct areas—a chemoceptive trigger zone and the vomiting center(1-3). The former structure is bilaterally situated at the surface of the medulla oblongata in the lateral region of the area postrema, and the vomiting center is located in the region of fasciculus solitarius and underlying reticular formation. Destruction of the trigger zone prevents vomiting from "central" acting drugs (apomorphine, morphine, Hydergine,‡ etc.) but does not affect vomiting after orally administered copper sulfate(4,5).

The observation that motion sickness in dogs(6) can also be prevented by destruction of the trigger zone raises the interesting possibility that certain apparently nonchemical emetic stimuli may actually have a chemical basis. It was of considerable interest, therefore, to determine whether the vomiting after irradiation might be similarly prevented by ablation of the trigger zone. Indirect evidence that this chemosensitive area is involved are the recent clinical reports on the relief of nausea and vomiting from irradiation with chlorpromazine(7), an apomorphine antagonist. The present experiments were designed to test the effect of surgical ablation of the trigger zone in preventing emesis in dogs after irradiation.

Methods. As controls, 33 normal mongrel dogs weighing 7 to 13 kg were fed commercial dog food and 30 minutes later irradiated with 300-800 r. A Picker deep therapy x-ray unit was employed with 260 KVP and 18 MA. The external filtration was 1 mm Al and 0.50

* This work was done as part of project for the U. S. Air Force.

† The authors wish to acknowledge the valuable assistance of George L. Sheldon and John Wilkinson.

‡ An equiproportional mixture of the methanesulfonates of dihydroergocornine, dihydroergocristine and dihydroergokryptine.