two have been determined. Tyrocidin causes a rapid hemolysis, the extent being proportional to the concentration of the lysin. Gramicidin causes a slower initial rate of hemolysis but this initial lag is followed by rapid destruction of red blood cells at a rate at least equal to the initial rate due to tyrocidin. Mixtures of the two components gave hemolysis patterns characteristic of the proportions of gramicidin and tyrocidin. (2) An alcohol-soluble substance or substances present in the wall of the red blood cell of the rat, possibly phospholipid in nature, combines with tyrocidin, and to a lesser extent with gramicidin, causing them to lose their hemolytic activity.

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Serum Cholinesterase Activity of Dogs in Shock or after Hepatectomy.* (19218)

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(Introduced by Jacob Fine.)
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Since the cholinesterase of the serum is probably derived in large part from the liver (1-3), its serum concentration may reflect hepatic function(4). Therefore, serum cholinesterase levels were measured as a part of a study of hepatic dysfunction in experimental hemorrhagic shock. For comparison these data were also obtained in hepatectomized dogs.

Methods. Hemorrhagic shock was produced in unanesthetized dogs, using the elevated reservoir bleeding technic previously described (5). Serum from arterial blood was obtained (a) before hemorrhage, (b) during hypotension before transfusion, (c) when hypotension recurred after blood replacement, and (d) just before death (Table I). Total hepatectomy was performed in 3 dogs by a one-stage method, preserving the integrity of the inferior vena cava(6). In one other dog the hepatic artery and portal vein were ligated and a portal-caval anastomosis was carried out without removal of the liver. In another, the hepatic artery alone was ligated. Serum from arterial blood was obtained before these procedures and at intervals thereafter. Serum cholinesterase and total esterase were determined colorimetrically, employing beta-carbonaphthoxy-choline iodide and beta-naphthyl acetate as the respective substrates

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TABLE I. Course of Hemorrhagic Shock.

Dog No.	Bleeding vol (cc/kg)	Period of hypotension* before transfusion (hr)	Period from transfusion to relapse† (hr)	Subsequent survival (hr)
1	50	1.6	2	1.6
2	61	2.6	.8	.8
3	57	4.3	.8	2
4	71	3	3.2	9
5	44	3	.8	.5
6	52	6.2	1.5	2
7	52	1.8	4.3	.2
8	67	6.5	1	—
9	44	3.5	1	—
10	47	5	—	recovered
11	60	8	—	,,
12	40	7.5	—	,,

* Arterial pressure 30 mm Hg.
† Post-transfusion relapse to arterial pressure 60 mm Hg.

TABLE II. Serum Cholinesterase Activity, Total Esterase Activity and Total Protein Concentration Before Bleeding (Control) and in Various Phases of Hemorrhagic Shock.

	Serum cholinesterase activity (units/100 cc)				Total esterase activity (units/100 cc)			
		Shock				Shock		
Dog No.	Control	1*	2†	3‡	Control	1*	2†	3‡
1	3	1.1	2.5	2.7	19.2	18.7	18.2	30
2	2.9	2.7	2	1.3	19.7	23.8	19.3	22.2
3	1.9	1.95	3	2.3	16.4	18	24.8	17.5
4	0	0	—	—	16.6	20.3	—	—
5	0	0	—	—	19.3	8.4	—	—
6	0	0	0	0	13.2	21	25.5	26.3
7	0	0	0	0	4.9	15.5	25.8	25.5
8	0	0	0	—	13.5	19.8	22.1	—
9	.6	1.9	1.6	—	21.5	26.4	36	—
10	3.1	2.6	—	—	15.3	21.2	—	—
11	2.4	2.9	—	—	16.1	19	—	—
12	2.8	1.1	—	—	14.9	15.1	—	—
Mean	*2.4*	*2*	*2.3*	*2.1*	*16.1*	*25.4*	*24.5*	*23.7*
	Mean total serum protein conc. (g/100 cc)				*6.5*	*6.7*	*6.6*	*6.7*

* 1. At end of period of controlled hypotension; before transfusion.
† 2. At post-transfusion relapse into shock.
‡ 3. At death (see Table I).

(7,8). One unit of activity is defined(7) as that amount of enzyme which liberates 10 mg of beta-naphthol per hour from the substrate under standard conditions.‡ Total protein concentration was determined by the copper sulfate falling-drop method(9).

Results. (Tables II and III). No change in serum cholinesterase activity was noted during hemorrhagic shock or during a 24-hour period following hepatectomy or hepatic devascularization. In 6 of the 17 experiments, the control specimen showed no serum cholinesterase activity and none appeared thereafter.

A rise in *total* esterase activity of the serum occurred in most of the dogs during shock or after hepatectomy. Serum total protein levels did not change significantly.

Discussion. At least 2 enzymes are adapted to the hydrolysis of acetylcholine. One of these is acetylcholinesterase in nervous tissue and in the red cell. The other is serum cholinesterase, which in the dog is found in serum, pancreas, parotid, parasympathetic ganglia and myenteric plexus. The substrate we employed is specific for serum cholinesterase since it is not hydrolyzed by acetylcholinester-

‡ 1/40 cc of dog serum is usually required. Hemolysis increases total esterase activity of the serum, but does not alter the serum cholinesterase activity as determined by this method.

TABLE III. Serum Cholinesterase Activity, Total Esterase Activity and Total Protein Concentration Before Operation (Control) and at Intervals After Hepatectomy or Hepatic Devascularization.

Dog No.	Preparation	Serum cholinesterase activity (units/100 cc) Control	Postoperative 4-6 hr	Postoperative 8-12 hr	Postoperative 16-24 hr	Total esterase activity (units/100 cc) Control	Postoperative 4-6 hr	Postoperative 8-12 hr	Postoperative 16-24 hr
H1	Hepatectomy	2.2	2.1	2.3	2.4	21.6	21.8	24.3	27.5
H2	"	.9	—	—	.9	16.2	—	—	19.4
H3	"	1.7	2.1	—	4.1	24.8	18	—	36.8
H4	Hepatic artery divided. Eck-fistula	4.6	4.4	4.4	4.4	23.1	22.5	—	27.4
H5	Hepatic artery divided.	0	—	—	0	28.5	—	—	29.4
Mean*		*1.4*	*2.7*	*3.3*	*2.6*	*21.4*	*20.8*	—	*27.4*
	Mean total serum protein conc. (g/100 cc)					*6.3*	*6.2*	*6.3*	*6.1*

* Does not include H5.

ase of nervous tissue or the red cell or by other aliesterases of serum(7).

The physiologic significance of serum cholinesterase is obscure. In the rat its activity can be reduced by feeding tri-ortho-cresyl phosphate to 20% of the normal level without noticeable physiologic effect(10). The average level in normal dogs was one-tenth the amount found by the same method in human serum(7), and in some apparently normal dogs none could be detected.§ The absence of a decline in serum cholinesterase activity during hemorrhagic shock or after hepatectomy suggests slow utilization of the enzyme. The blood level of this enzyme, therefore, is not a sensitive index of acute changes in hepatic function in the dog. The findings are in agreement with those of an earlier study of serum cholinesterase activity during hemorrhagic shock in the dog(11), and with the delayed fall in serum cholinesterase in rats following acute liver damage produced by carbon tetrachloride(3). Guinea pig serum cholinesterase activity is reported to decline following trauma(13). In these earlier studies acetylcholine was used as substrate, so that serum cholinesterase alone was not specifically measured.

Plasma fibrinogen concentration does not fall during hemorrhagic shock or after hepatectomy in the dog(14). On the other hand, the prothrombin activity of dog plasma declines in proportion to the degree of deterioration during shock(15).

The administration of cholinesterase to dogs in hemorrhagic shock has been found ineffective(11). A reported favorable effect of cholinesterase given to dogs subjected to intestinal trauma cannot be evaluated in terms of an effect upon shock because the test dogs were also under the influence of thyroid hormone and barbiturate anesthesia(11).

The total esterase activity of the serum toward beta-naphthyl acetate includes the activity of ali-esterases(7), cholinesterases(7), and lipases(16) arising from pancreas, liver, blood cells and other tissues. No explanation is apparent for the rise in total esterase activity.

Conclusions. (1) Serum cholinesterase activity of normal dogs is approximately one-tenth that of normal human serum as measured by a colorimetric method employing beta-carbonaphthoxy-choline iodide as substrate. (2) No change occurs in the serum cholinesterase activity of dogs during hemorrhagic shock, or during 20 hours of survival after hepatectomy.

§ Technics using acetylcholine as substrate do not yield such low values in dog serum(11,12).

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Further Studies on Formate Incorporation by Leukemic Blood Cells.* (19219)

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It has been observed that at one hour after injection of C^{14}-formate into mice with advanced Ak-4 leukemia, the greater portion of the leukemic prolymphocytes give positive autoradiograms on nuclear track emulsion while very few normal-appearing lymphocytes and practically none of the polymorphonuclears or erythrocytes are positive(1). Two preliminary explanations of these results were considered: (a) that the C^{14}-formate was incorporated during the anabolic phases of cell division and that the leukemic cells were dividing very rapidly as compared to normal cells, or (b) that the formed leukemic cells were metabolizing formate much more rapidly than were normal blood elements. The present study was designed to obtain information which might shed light on this question. In these experiments, leukemic blood has been exposed to C^{14}-formate away from sites of hematopoiesis for subsequent autoradiographic examination.

Experimental. Blood was aspirated from the heart of Akm mice with advanced transplanted Ak-4 leukemia (a rather acute lymphoid strain). This blood was heparinized and placed in a small cellophane bag which was then introduced through an incision into the abdominal cavity of leukemic mice (Ak-4). These mice (with leukemic blood-containing cellophane bags) were injected IP. with 100 microcuries of C^{14}-formate. After one hour, the mice were sacrificed and blood smear autoradiograms were prepared on cardiac and cellophane-bag blood after each had been washed twice with inactive sera. The details of the procedure employed in making the autoradiograms have been presented(1). A number of blood smears on NTB plates (10 μ emulsion thickness) were made on each sample. The plates were developed for 2 or 20 minutes after exposure periods in the dark from 1 to 10 weeks. All of the NTB plates were examined under the microscope and a hundred or more cells on each were classified as grossly positive or negative. The results of these experiments are summarized in Table I.

Discussion. The white blood counts on the leukemic host animals in Exp. No. 1 and 2 were 7,500 and 34,800, respectively, somewhat

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