record was kept of body weight and food consumption. Diets used were the same as previously described[4] except for the addition of vitamin K (2-methyl-1,4-naphthoquinone, 5 mg/kg) and vitamin E (a-tocopherol, 50 mg/kg). The diet containing 1 mg thiamine and 0.75 g choline per kilo of food gives optimal growth at 68°F, while for the same growth rate at 90-91°F 2 mg thiamine and 5 g choline are needed. The following results were obtained after 5 weeks of fresh daily feedings of these diets to 3 series of rats in the heat and cold:

	Wt gain 3rd, 4th, 5th wks, g	Food eaten in same period, g
Optimal cold room diet:		
Rats kept at 68° F	72.50 ± 4.16	284
Rats kept at 90-91°	42.50 ± 2.12	169
Opt. hot room diet:		
Rats kept at 90-91°F	70.83 ± 1.54	201

The difference between the 2 groups in the hot room (28.33 ± 2.70) would almost never occur by chance alone. This sharply reduced rate of growth, seen when the cold-room diet is used for rats kept in the heat, agrees with all our former results obtained with weekly or semi-weekly feedings. It therefore cannot be vitamin loss on standing exposed to tropical warmth which accounts for the higher thiamine and choline needs in this environment.

Until carefully controlled and adequately planned investigation has shown that thiamine and choline requirements *are not increased* in hot environments, it would seem that our positive findings in the matter should not be dismissed.

[4] Mills, C. A., *Arch. Biochem.*, 1943, **2**, 159.

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The Presence of a Choline-like Substance in Several Injectable Solutions of Liver.*

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It has been shown that experimental polycythemia may be depressed in dogs by the feeding of raw liver or the injection of a purified solution of liver,[1] and also by the feeding of choline hydrochloride.[2] Jacobs[3] has reported that a crude solid liver extract which is effective in the treatment of pernicious anemia contains at least 1% of choline. We have reported the presence of choline in Stomach U.S.P. and the effectiveness of the latter in reducing experimental polycythemia in dogs.[4]

The purpose of this investigation was to determine whether highly purified parenteral solutions of liver contain any choline or choline-like activity.

Methods. Seven injectable solutions of liver, which are available in the market, and which bear labels giving their anti-pernicious anemia potencies in U.S.P. units were obtained. These were tested for possible vasodepressor activity on dogs anesthetized by pentobarbital sodium (30 mg/kg). The extracts were injected intravenously, and the mean carotid arterial pressure was recorded from a mercury manometer.

In further experiments, 6 of the liver extracts were acetylated by the method of Mentzer *et al.*[5] modified to the extent that acetic anhydride was used instead of acetyl chloride. Choline hydrochloride when acetyl-

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[1] Davis, J. E., *Am. J. Physiol.*, 1938, **122**, 397.

[2] Davis, J. E., *Am. J. Physiol.*, 1939, **127**, 322.

[3] Jacobs, H. R., *J. Lab. and Clin. Med.*, 1938, **24**, 128.

[4] Davis, J. E., in press.

[5] Mentzer, C., Corteggiani, E., and Carayon-Gentil, A., *Bull. Soc. Chim. Biol.*, 1939, **21**, 503.

TABLE I.

Changes Produced in the Mean Carotid Arterial Blood Pressure by the Intravenous Injection of Various Liver Extracts. Pressure changes in millimeters of mercury are recorded as rise (+) or fall (—), or no change (0). Dosages of extracts are given in U.S.P. units (u).

Preparation of liver solution Units per cc	Untreated liver solution	Acetylated liver solution	After atropine: Untreated liver extr.	After atropine: Acetylated liver extr.
I. 20	0 (5u)	— 18 (3u)		0 (3u)
II. 15	0 (5u)	— 60 (7.5u) — 49 (5u) — 23 (3u) — 13 (1u) — 12 (2u)		0 (7.5u) 0 (3u)
III. 10	0 (2.5u)	— 30 (2.5u) — 76 (4u) — 51 (1.6u)		0 (2.5u) +9 (4u)
IV. 5	0 (5u)	— 70 (8u) — 26 (7u)		0 (8u) 0 (7u)
V. 4	—80 (6u)	— 90 (1u) — 90 (2u)	—72 (6u)	—15; +20 (1u) —26; +44 (2u)
VI. 2	—48 (0.4u)	—110 (0.8u)	—63 (2u)	—38 (1u)
VII. 1	—44 (1u)	not tested	—27 (1u)	not tested

ated by this method showed the same vasodepressor activity as pure acetylcholine in similar concentration. The acetylated extracts were tested for vasodepressor activity both before and after atropinization of the animals.

Results. Table I shows that the 4 most potent preparations (*i.e.*, containing 5 or more U.S.P. units per cc according to their labels) produced no significant change in blood pressure when injected intravenously into dogs in doses of 2.5 to 5 units. After acetylation, however, these same extracts caused significant reductions of blood pressure. No fall of blood pressure occurred when these acetylated extracts were given after the intravenous injection of atropine sulfate (0.5 mg per kg).

The other 3 preparations, with labeled antianemic potencies of less than 5 units per cc, caused marked decreases of blood pressure following the intravenous injection of even small doses (Table I). The vasodepressor activity of 2 of these extracts was tested after acetylation, and appeared to be increased thereby. Atropinization of the animals inhibited or greatly decreased the vasodepressor activity of these 2 extracts. In one experiment the injection of 2 mg of acetylcholine after atropine, caused an exact duplication of the blood pressure record resulting from the injection of 1 unit of acetylated solution number 5 (brief fall of 15 mm followed by a rise to 20 mm above normal).

Extracts Nos. 5, 6 and 7 (untreated) caused significant decreases in blood pressure when injected after atropinization of the animals.

Fig. 1 shows blood pressure tracings which illustrate the kind of records obtained in these experiments.

Discussion. No real correlation was found to exist between antianemic unitage and blood pressure lowering activity in these extracts except perhaps in one experiment where acetylated extracts Nos. 1, 2, and 3 given in doses of 2 or 3 units produced somewhat comparable falls in pressure.

The fact that acetylation produced a blood pressure lowering action in extracts 1-4, and increased that activity already present in the others, and that the action is abolished or diminished by atropine—leads us to believe that choline or a choline-like substance is present in all of the liver extracts tested.

That the acetylation of substances other than choline can result in a product with

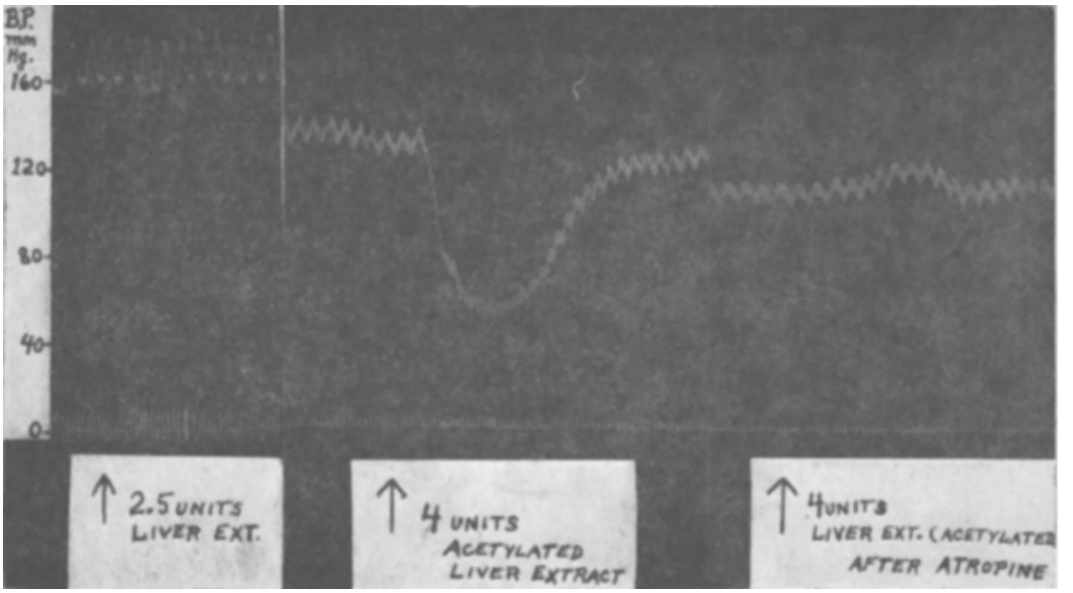

FIG. 1.
Typical Blood Pressure Record Following the Intravenous Injection of Untreated and Acetylated Liver Extracts.

Tracing at left shows the effect of 0.25 cc of a brand of purified solution of liver upon the blood pressure of a dog. The same brand of liver extract (Number III; Table I) was acetylated and injected into another dog both before and after atropinization. Time is recorded in seconds.

increased vasodepressor action has been shown by Hunt[6] and by Dale.[7] However, few such substances are known to be present in the animal body, most of them being products of laboratory synthesis.

Conclusion. Six injectable liver extracts were found to contain choline or a choline-like substance, since their acetylation produced vasodepressor activity which was antagonized by atropine.

[6] Hunt, R., *J. Pharm. and Exp. Therap.*, 1914, **6**, 477.

[7] Dale, H. H., *J. Pharm. and Exp. Therap.*, 1914, **6**, 147.

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Phosphatase Activity of Human Erythrocytes.

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Evidence has accumulated which indicates that phosphatases occur in the erythrocytes of dog, beef, horse, rabbit, rat, and man. To provide further information about phosphomonoesterase occurring in the normal human red blood cells the enzymatic activity of red cell hemolysates was studied as to (1) the presence of alkaline and acid phosphatase, (2) the effect of fluoride and magnesium upon the enzymatic activity, (3) the relation of erythrocyte phosphatase to plasma phosphatase.

Procedure. The erythrocytes were washed 3 times with normal saline. The washed cells obtained from x cc of oxalated or heparinized blood were hemolyzed by adding distilled