when high titer interferon and highly sensitive mouse cells were employed(11).

Summary. A TRIC agent (LB-1), a member of the psittacosis-LGV-trachoma group, can induce the production *in vivo* and *in vitro* of a material with antiviral activity similar to virus-induced interferon. The interferon induced in cell culture by LB-1 had a molecular weight of about 50,000 and hence appeared to differ significantly from virus-induced interferon prepared in cell culture or *in vivo*.

ADDENDUM: The peak of TRIC-induced interferon production in serum of mice is late (6-13 hours), similar to that of virus(3), brucella(3), or statalon(10), but distinct from the early (2 hour) peak appearing after endotoxin(4).

1. Isaacs, A., Adv. Virus Res., 1963, v10, 1.
2. Hopps, H., Kohno, S., Kohno, M., Smadel, J. E., Bact. Proc., 1964, 115.
3. Youngner, J. S., Stinebring, W. R., Science, 1964, v144, 1022.
4. Stinebring, W. R., Youngner, J. S., Nature, 1964, v204, 712.
5. Wheelock, E., Science, 1965, v149, 310.
6. Kleinschmidt, W., Cline, J., Murphy, E., Proc. Nat. Acad. Sci., Wash., 1964, v52, 741.
7. Mordhorst, C. H., Reinecke, V., Acta Path. Microbiol. Scand., 1965, v65, 545.
8. Jawetz, E., Ann. Rev. Microbiol., 1964, v18, 301.
9. Hallum, J. V., Stinebring, W. R., Youngner, J. S., Virology, 1965, v27, 429.
10. Merigan, T. C., Kleinschmidt, W. J., Nature, 1965, v208, 667.
11. Hanna, L., Merigan, T. C., Jawetz, E., Proc. Soc. Exp. Biol. & Med., 1966, v122, 417.
12. Gordon, F. B., Quan, A. L., Trimmer, R. W., Science, 1960, v131, 733.
13. Padgett, B. L., Walker, D. L., Proc. Soc. Exp. Biol. and Med., 1962, v111, 364.
14. Merigan, T. C., Winget, C. A., Dixon, C. B., J. Mol. Biol., 1965, v13, 679.
15. Naficy, K., Ann. N. Y. Acad. Sci., 1965, v130, 449.
16. Shope, R. C., J. Exp. Med., 1953, v97, 601.
17. Ho, M., Bact. Rev., 1964, v28, 367.
18. Merigan, T. C., Science, 1964, v145, 811.
19. Ruiz-Gomez, J., Isaacs, A., Virology, 1963, v19, 8.

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Blood Preservation Solutions Containing Adenine, Phosphate, and Guanosine.* (31152)

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Although ACD (acidified citrate dextrose) solution has been in routine use for blood storage since World War II, there have been suggestions that this medium could be improved by addition of purine nucleosides such as adenosine or inosine (see review in ref. 1, also 2,3). Addition of adenine, with or without inosine, was also observed to have a beneficial effect(4,5,6). Other suggested alterations in the formulation include addition of phosphate(7,8) or control of pH(9,10). Added guanosine might be beneficial as an energetic purine nucleoside(11) or might have some additional role perhaps as a structural component(12). The present study embodies many suggestions for the improvement of ACD and emerges with what appears on the basis of *in vitro* testing to be an improved blood banking solution.

Methods and materials. The criteria for improved red cell storage media were prolonged maintenance of ATP(13,14) and nucleotide adenine (AMP + ADP + ATP). The latter measures the adenine pool available for ATP formation(15). Because of donor variability in ATP levels, the general plan of each experiment was to collect 100 ml of blood successively from the same donor into 5 vacuum bottles. These bottles† contained the various formulations to be tested

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† Baxter H-18 Plasma-Vac, 200 ml capacity, courtesy of Dr. L. D. Bechtol, Baxter Laboratories, Inc., Morton Grove, Ill.

and had been autoclaved. Solutions were adjusted such that the pH of the collected blood would be about 7.0. The collected blood was stored in our Blood Bank,‡ samples being withdrawn aseptically as needed. Nucleotides were assayed by column chromatography(16). The *final* concentration of the formula compounds in the collected blood is shown under each Figure.

Results. Phosphate. In Fig. 1A are shown the results of the experiment utilizing the CPD (citrate-phosphate-dextrose) formulations of Gibson(8) but with adenine added. The 5th bottle, containing a much higher phosphate concentration and heparin instead of citrate, showed better maintenance of ATP and nucleotide adenine. In fact, the results after 3 weeks were slightly superior to the initial findings.

Citrate levels. Some of our earlier results (17) had suggested that citrate might have a deleterious effect on red cell metabolism (and hence on ATP level). Fig. 1B shows the results of an experiment set up with heparin as an anticoagulant and citrate at various levels (ACD has a final citrate concentration of about 14 mM). No harmful effect of the citrate level was evident, but in these formulations the phosphate level was very high in contrast to ACD which has no phosphate. Adenine was also present.

Heparin, phosphate, adenine. In the experiment depicted in Fig. 1C, the first bottle contained phosphate at a high level, adenine, and heparin, in addition to citrate and glucose. Omission of the heparin in Bottle 2 had little effect and reducing the phosphate level to 9 mM in Bottle 3 had only a slight effect. Reducing the phosphate level to 2 mM (Bottle 4) or omitting the adenine (Bottle 5) had a marked effect on ATP level.

Guanosine. In the experiment of Fig. 1D, guanosine was beneficial in a citrate system containing high phosphate, adenine and glucose (Bottle 4 *vs* 3) and perhaps less so with heparin replacing citrate (Bottle 2 *vs* 1).

Guanosine, longer storage period. Fischer (18) has claimed that citrated blood with added inosine, adenine, and guanosine can be stored 42 days. The results shown in Fig. 1F (Bottle 5) support this claim. The possibility of improving the formulations by adding heparin (Bottles 2 and 4) are slight. A lower phosphate level (9 mM, Bottle 5, *vs* 18 mM, Bottle 3) is somewhat better (and more desirable for transfusion).

ACD vs *citrate-glucose-phosphate-adenine-guanosine.* Results of such a comparison are shown in Fig. 1F, Bottle 1 *vs* 2 (the phosphate concentration is intermediate between the 18 and 9 mM levels used previously). The need for phosphate is shown by the poorer results when it was omitted (Bottle 3). Adenine is also needed (Bottle 4). The omission of guanosine (Bottle 5) is much less serious, but these results are in blood stored 21 days. The beneficial results of guanosine may be evident only in longer times.

Inosine, adenine, guanosine. The results in Fig. 1G indicate that at 6 weeks, ACD (Bottle 1), ACD plus adenine (Bottle 2) and CPD (Bottle 3) are not very good. Addition of adenine, inosine, and guanosine without phosphate leads to a higher ATP level (Bottle 4). Adenine, high phosphate, and guanosine are an even better combination (Bottle 5).

Phosphate levels, citrate levels, inosine. The results shown in Fig. 1H indicate that in this combined formulation, 15 mM phosphate (Bottle 3) is not much better than 10 mM phosphate (Bottle 1), but that 5 mM phosphate (Bottle 4) may be slightly inferior. Lowering the citrate level (Bottle 2) may help a little (no clotting was observed in this bottle). The addition of inosine (Bottle 5) had no beneficial effect, but there is evidence from other sources that inosine contributes an effect in blood stored longer than the 4 weeks of this experiment.

Discussion. The standard method of collecting blood in ACD and storing it at 4° for 21 days has remained unchanged for the last 20 years with the possible exception that plastic bags are gradually replacing glass bottles. Even this change is dictated as much by convenience and lower cost of storing empty containers as by demonstrated superiority in preservation. The ACD solution is

‡ These studies were greatly facilitated by the cooperation of the personnel of the Blood Bank at Buffalo General Hospital and its Director, Dr. James F. Mohn.

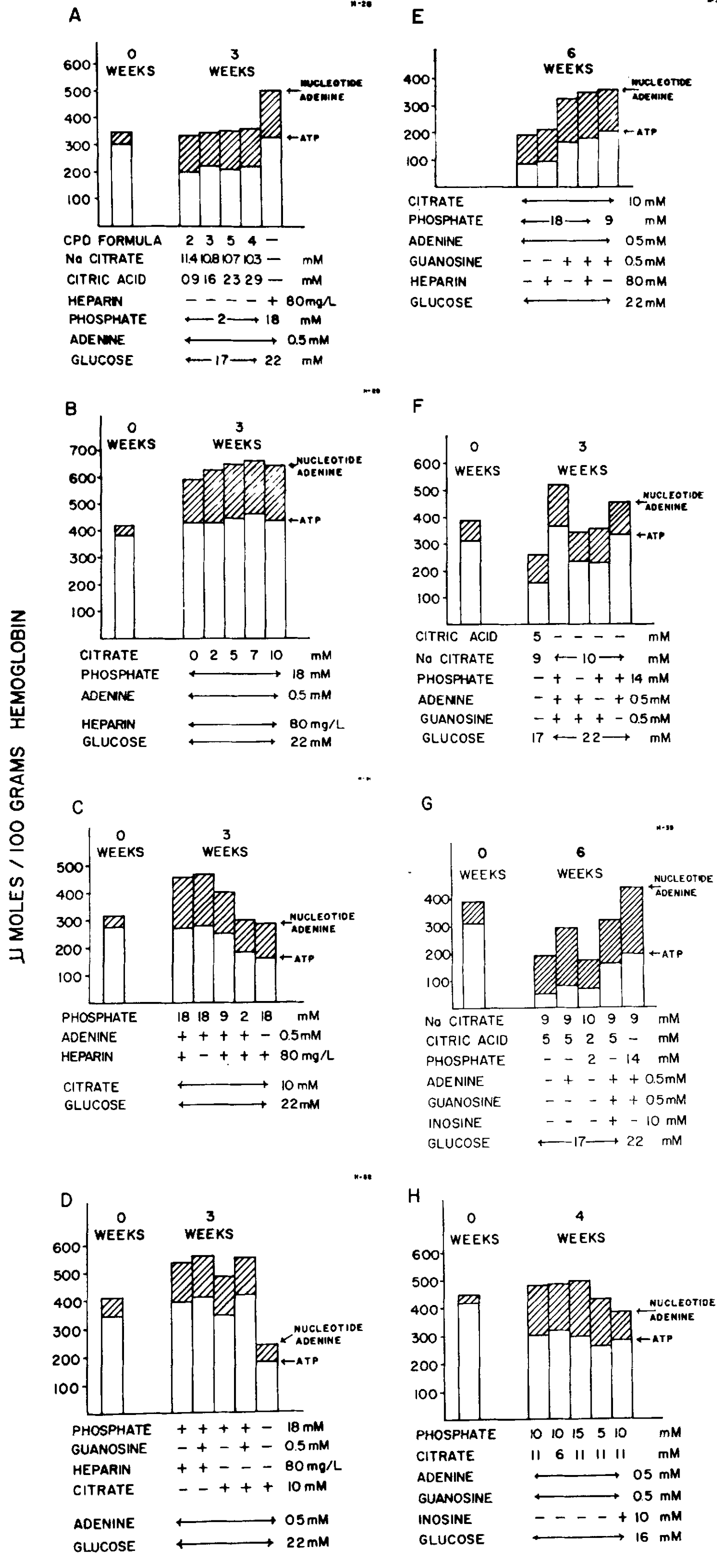
µ MOLES / 100 GRAMS HEMOGLOBIN
A
0 WEEKS
3 WEEKS
NUCLEOTIDE ADENINE
ATP
600
500
400
300
200
100
CPD FORMULA 2 3 5 4 —
Na CITRATE 11.4 10.8 10.7 10.3 — mM
CITRIC ACID 0.9 1.6 2.3 2.9 — mM
HEPARIN — — — — + 80mg/L
PHOSPHATE ←2→ 18 mM
ADENINE ← → 0.5 mM
GLUCOSE ←17→ 22 mM
E
6 WEEKS
NUCLEOTIDE ADENINE
ATP
400
300
200
100
CITRATE ← → 10 mM
PHOSPHATE ←18→ 9 mM
ADENINE ← → 0.5mM
GUANOSINE — — + + + 0.5mM
HEPARIN — + — + — 80mM
GLUCOSE ← → 22mM
B
0 WEEKS
3 WEEKS
NUCLEOTIDE ADENINE
ATP
700
600
500
400
300
200
100
CITRATE 0 2 5 7 10 mM
PHOSPHATE ← → 18 mM
ADENINE ← → 0.5 mM
HEPARIN ← → 80 mg/L
GLUCOSE ← → 22 mM
F
0 WEEKS
3 WEEKS
NUCLEOTIDE ADENINE
ATP
600
500
400
300
200
100
CITRIC ACID 5 — — — — mM
Na CITRATE 9 ←10→ mM
PHOSPHATE — + — + + 14 mM
ADENINE — + + — + 0.5mM
GUANOSINE — + + + — 0.5mM
GLUCOSE 17 ←22→ mM
C
0 WEEKS
3 WEEKS
NUCLEOTIDE ADENINE
ATP
500
400
300
200
100
PHOSPHATE 18 18 9 2 18 mM
ADENINE + + + + — 0.5mM
HEPARIN + — + + + 80 mg/L
CITRATE ← → 10 mM
GLUCOSE ← → 22mM
G
0 WEEKS
6 WEEKS
NUCLEOTIDE ADENINE
ATP
400
300
200
100
Na CITRATE 9 9 10 9 9 mM
CITRIC ACID 5 5 2 5 — mM
PHOSPHATE — — 2 — 14 mM
ADENINE — + — + + 0.5mM
GUANOSINE — — — + + 0.5mM
INOSINE — — — + — 10 mM
GLUCOSE ←17→ 22 mM
D
0 WEEKS
3 WEEKS
NUCLEOTIDE ADENINE
ATP
600
500
400
300
200
100
PHOSPHATE + + + + — 18 mM
GUANOSINE — + — + — 0.5 mM
HEPARIN + + — — — 80 mg/L
CITRATE — — + + + 10 mM
ADENINE ← → 0.5mM
GLUCOSE ← → 22mM
H
0 WEEKS
4 WEEKS
NUCLEOTIDE ADENINE
ATP
600
500
400
300
200
100
PHOSPHATE 10 10 15 5 10 mM
CITRATE 11 6 11 11 11 mM
ADENINE ← → 0.5 mM
GUANOSINE ← → 0.5 mM
INOSINE — — — — + 10 mM
GLUCOSE ← → 16 mM

FIG. 1. Effect of various additives on levels of ATP and nucleotide adenine (AMP + ADP + ATP) in blood stored several weeks at 4°C. All concentrations are expressed as millimolarity in final collected blood.

quite acid (pH 4.5-5.5, by law), "hyperglycemic," with a high concentration of a relatively foreign compound (citrate), and of a tonicity which first shrinks the red cells and then allows the cells to swell unnaturally during storage. The present experiments show that from a metabolic point of view, the standard ACD formulation can be greatly improved by adding such compounds as adenine, guanosine, phosphate, and perhaps inosine, and by modifying the pH of the collecting solution so the pH of the collected blood will be closer to a normal blood pH. From the foregoing experiments, a formulation that seems worthy of testing by red cell survival is as follows (all concentrations given as final millimolarity in collected blood): citrate-12; glucose-17; phosphate-5; adenine-0.5; guanosine-0.5; and possibly inosine-10. The final pH after collection of blood should be no lower than 7.0. The latter requirement stems from the observation that glycolysis decreases with falling pH(19), perhaps by affecting the hexokinase reaction. The decrease in glycolytic rate during storage is not due to the increasing concentration of lactate but to the increasing H^+ concentration(20).

There are some practical difficulties associated with formulations of the type recommended here. The purine compounds are not inexpensive and they are generally not very soluble. If used in high concentration, such as 10 mM inosine, they might supply an appreciable extra purine load that would be converted to uric acid in the body. Phosphate at the level suggested may seem to risk an unnecessary load in a transfusion situation, but no definite experiments have been done to determine just what does happen in this case.

The present study concentrates on only certain metabolic criteria and largely ignores tonicity effects or possible damage to the red cell membrane. These aspects must await future studies.

Summary. Blood from the donor was drawn into 5 successive bottles, each containing varying amounts of citrate, heparin, glucose, phosphate, adenine, guanosine, and inosine. The blood was stored under blood bank conditions for up to 6 weeks and samples were aseptically withdrawn at intervals for column chromatographic assay of ATP and nucleotide adenine (AMP + ADP + ATP). Phosphate was important in maintaining ATP and nucleotide adenine but at least 5 mM (final concentration in collected blood) was required. Adenine (0.5 mM) was as effective as phosphate, and the effect of the two was additive. Heparin, especially in the presence of phosphate, was not markedly superior to citrate in its effect on red cell metabolism. Citrate could be used at half the concentration in ACD without apparent clotting. Guanosine (0.5 mM) was definitely beneficial at 6 weeks, although without much apparent effect at 3 weeks. Inosine did not improve results in 4 weeks and was not tested at 6 weeks.

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1. Bishop, C., in the Red Blood Cell, C. Bishop, D. M., Surgenor, eds., Academic Press, New York, 1964, p173.
2. Gabrio, B. W., Donohue, D. M., Huennekens, F. M., Finch, C. A., J. Clin. Invest., 1956, v35, 657.
3. Lange, R. D., Crosby, W. H., Donohue, D. M., Finch, C. A., Gibson, J. G., ibid., 1958, v37, 1485.
4. Nakao, M., Nakao, T., Arimatsu, Y., Yoshikawa, H., Proc. Japan Acad., 1960, v36, 43.
5. Simon, E. R., Chapman, R. G., Finch, C. A., J. Clin. Invest., 1962, v41, 351.
6. Bishop, C., Transfusion, 1964, v4, 265.
7. Parpart, A. K., Gregg, J. R., Lorenz, P. B., Parpart, E. R., Chase, A. M., J. Clin. Invest., 1947, v26, 641.
8. Gibson, J. G., Rees, S. B., McManus, T. J., Scheitlin, W. A., Am. J. Clin. Path., 1957, v28, 569.
9. Bishop, C., Transfusion, 1962, v2, 408.
10. Beutler, E., Duron, O., ibid., 1965, v5, 17.
11. Prankerd, T. A. J., Lancet, 1956, v1, 469.
12. Fischer, H., Fol. Haematol., 1962, v78, 356.
13. Gabrio, B. W., Finch, C. A., Huennekens, F. M., Blood, 1956, v11, 103.
14. Nakao, K., Wada, T., Kamiyama, T., Nakao, M., Nagano, K., Nature, 1962, v194, 877.
15. Bishop, C., J. Biol. Chem., 1960, v235, 3228.
16. Bishop, C., Rankine, D. M., Talbott, J. H.,

ibid., 1959, v234, 1233.
17. Bishop, C., Transfusion, 1963, v3, 263.
18. Fischer, H., German Patent No. 1141747, 1963.
19. Kashket, S., Rubinstein, C., Denstedt, O. F., Gosselin, S. M., Can. J. Biochem. Physiol., 1957, v35, 827.
20. Bishop, C., Transfusion, 1962, v2, 256.

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Protection Against Histamine Shock by Catecholamines in *Bordetella pertussis*-Treated, Adrenalectomized, or Adrenergic Blocked Mice. (31153)

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The intraperitoneal (i.p.) LD_{50} of histamine for normal mice is between 750-1000 mg/kg body weight. This remarkable resistance to histamine can be decreased in some strains by treatment with the histamine sensitizing factor (HSF) from *Bordetella pertussis,* by removal of the adrenal glands(1,2), or by administration of β-adrenergic blocking agents, such as dichloroisoproterenol (DCI) and nethalide(3). The increased sensitivity is not limited to histamine, since pertussis vaccine and adrenalectomy also increase the susceptibility of mice to serotonin, anaphylactic shock, cold shock and various other forms of stress(2). In addition to their potentiating effects on histamine sensitivity, β-adrenergic blocking agents also increase the susceptibility of mice to serotonin(3).

The exact mechanism by which this hypersensitization phenomenon is brought about is still not completely known. The characteristics of hypersensitization after adrenalectomy or treatment with HSF are quite similar, suggesting that adrenal hormones might be involved in the hypersensitization produced by HSF. Adrenalectomized mice can be protected from histamine shock with cortisone and epinephrine(4,5) and adrenalectomized rats can be protected by epinephrine alone(6). HSF-treated mice have also been protected from histamine shock with large doses of cortisone(7,8) but we have found no report of protection with catecholamines; to the contrary, failure to protect *B. pertussis*-treated mice with epinephrine has been reported(9).

During anaphylaxis and histamine shock in the mouse, there is a profound loss of blood volume as shown by a marked rise in hematocrit values(4,5,9-12). Hypotension is known to be a potent stimulus for the release of endogenous catecholamines in the dog(13). The salutary effects of these hormones are presumably due to their vasopressive actions which improve venous return to the heart and help maintain circulation to vital organs, *e.g.*, heart and brain. The purpose of this investigation was to determine whether catecholamines could protect histamine hypersensitive mice against histamine shock.

Materials and methods. Mice. Equal numbers of 7-8-week-old male and female CFW mice raised in our laboratory were used. During the experimental period, these mice were housed in groups of 5 in glass jars, 7 inches deep by 7 inches in diameter, containing beet pulp bedding and Purina Laboratory Chow. Sexes were kept separated.

Histamine sensitizing factor. HSF was given as a dialyzed and lyophilized alkaline saline extract (SE) of acetone-dried *B. pertussis* cells(14).

Drugs. L-epinephrine, dl-epinephrine, dl-norepinephrine-HCl (dl-arterenol-HCl), l-ephedrine alkaloid and dl-ephedrine HCl were obtained from Sigma Chemical Co. Histamine was given as histamine diphosphate (Nutritional Biochemicals Corp.). Dichloro-