what contradicts the hypothesis that vit. B_{12} is concerned in the conversion of PGA to CF (21) for, if such were true, CF should be considerably more active than PGA on a weight for weight basis.

Summary. Exposure of citrovorum factor (leucovorin) solutions to pH 2 for 24 hours at 25°C resulted in inactivation of *L. citrovorum* activity but produced no alteration in the activity for *S. faecalis*. The acid-treated leucovorin was about one-tenth as active as leucovorin in reversing Aminopterin toxicity in mice. When administered to patients with pernicious anemia in relapse the acid-treated leucovorin was less active than leucovorin.

Received October 15, 1951. P.S.E.B.M., 1951, v78.

Effect of Hypoxemia on Edema Formation in Perfused Isolated Rat Hind Limb.* (19142)

EDITH L. DI PASQUALE AND ALFRED A. SCHILLER.

From the Department of Physiology, University of Illinois College of Medicine, Chicago.

The effect of hypoxemia on capillary function has been the object of numerous investigations since Landis(1) noted that complete anoxemia caused a fourfold increase in capillary filtration rate in the frog mesentery. Maurer(2,3) used the rate of flow and protein content of the cervical lymph in dogs to indicate a change in permeability of the capillaries during respiratory hypoxemia. In all cases lymph flow increased steadily with progressing hypoxemia to as low as 7 volumes % oxygen content. However, this change cannot be attributed to hypoxemia alone when considering that many other factors affect the overall flow of lymph. Warren and Drinker(4) studied the lung lymph flow in the same manner using a level of hypoxemia as low at 4.4 vol. %, and found that lung lymph flow consistently increased. Further indirect evidence that graded hypoxemia causes graded increases in capillary permeability was reported by Pochin (5), who occluded the blood vessels at the base of the rabbit's ear for 2 to 24 hours and noted an increase in the thickness of the ear in proportion to the time of occlusion. Hopps and Lewis(6) found that anoxemia did not facilitate the passage of antibody globulin nor of T-1824 through the vascular epithelium of the guinea pig. McMichael and Morris(7) as well as Henry *et al.*(8) utilized the procedure of venous congestion of the upper extremities of human beings. The former authors found no increase in swelling of the arm at 9.5 vol. % oxygen content and the latter no change in calculated loss of protein from the blood of the congested arm at 60 to 70% oxygen saturation of the blood. However, at 15 to 20% saturation or 4 to 6 vol. % oxygen content a significant loss of protein occurs(9). Stead and Warren(10) noted that in patients with chronic emphysema in whom the blood was 50 to 60% saturated, no signs of protein "leakage" were present. Perfusion technics in the frog were used by Danielli(11) and Saslow(12) with different interpretations

* Aided by a grant from the National Heart Institute of the National Institutes of Health, U. S. Public Health Service.

1. Landis, E. M., *Am. J. Physiol.*, 1928, v83, 528.
2. Maurer, F. W., *Am. J. Physiol.*, 1940, v131, 331.
3. Maurer, F. W., *Am. J. Physiol.*, 1941, v133, 170.
4. Warren, M. F., and Drinker, C. K., *Am. J. Physiol.*, 1942, v136, 207.
5. Pochin, E. E., *Clin. Sci.*, 1939-42, v4, 341.
6. Hopps, H. C., and Lewis, J. H., *Am. J. Path.*, 1946, v22, 656.
7. McMichael, J., and Morris, K. M., *J. Physiol*, 1936, v87, 74P.
8. Henry, J. P., Klain, I., Movitt, E., and Meehan, J., *Fed. Proc.*, 1946, v5, 44.
9. Henry, J. P., Goodman, J., and Meehan, J., *J. Clin. Invest.*, 1947, v26, 1119.
10. Stead, E. A., and Warren, J. V., *J. Clin. Invest.*, 1944, v23, 283.
11. Danielli, J. F., *J. Physiol.*, 1940, v98, 109.
12. Saslow, G., *Am. J. Physiol.*, 1938, v124, 360.

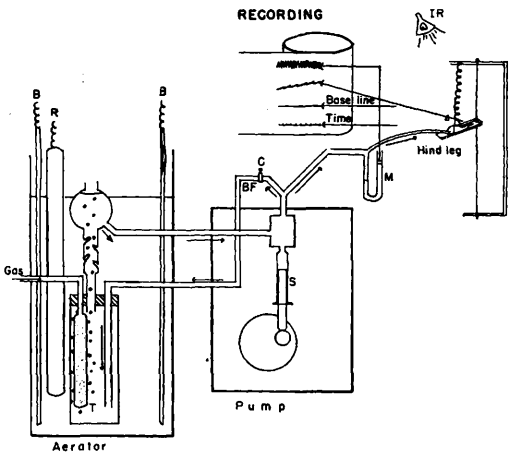

FIG. 1. Perfusion apparatus. T, scintered glass tube; B, heating blades; R, thermoregulator; C, screw clamp; BF, backflow tube; S, syringe; M, mercury manometer; IR, infra red lamp. Description in text.

of the effectiveness of using red blood cells in the perfusion medium to prevent edema. Saslow attributes the beneficial effect of red blood cells to their oxygen carrying capacity whereas Danielli concluded that in addition to the availability of oxygen the presence of platelets in blood perfusates was also necessary to clog the "pores" in the capillary endothelium and thereby prevent edema. It is apparent from the above that a consensus is lacking concerning the effect of hypoxemia on capillary permeability, especially within the physiological range.

In the following investigation a modification of the method of Hyman(13) was chosen to study quantitatively the effect of hypoxemia on capillary function. It consists of perfusing isolated rat hind limbs and determining the rate of edema formation by recording continuously the weight of the perfused leg.

Methods. The perfusion apparatus (Fig. 1) contains the aerators designed on the principle of the Clark *et al.*(14) oxygenator. A methylpolysiloxane resin, Dow Corning Antifoam A, is used to coat the inside surface of the aerators. A scintered glass aerating tube disperses the gas into fine bubbles and also serves as a stirring mechanism. The reservoirs are in a water bath maintained at 41°C by a thermoregulator operating 2 heating blades and a stirrer to ensure the delivery of fluid to the hind limb at 37°C. Separate reservoirs are used for the hypoxic and for the oxygenated perfusion fluids. Variations in oxygen content are achieved by bubbling 100% oxygen for at least 1 hour, or 100% nitrogen that has been bubbled through alkaline pyrogallol to remove any oxygen(15). Three-way stopcocks connecting both reservoirs with the perfusion pump permit a rapid change of fluids. The perfusion pump consists of an automatic pipetting machine to which a 3 cc siliconed syringe is attached with the barrel fixed at the Luer-Lok tip and the plunger attached to a cam which pushes or pulls the plunger as it rotates eccentrically. The continually rotating cam provides a pulsatile flow of fluid at a rate of 40 strokes per minute. On the downstroke of the plunger, fluid enters the syringe from the aerators. On the upstroke fluid is pumped through a valve assembly into a Y-tube. One branch of the Y, a backflow tube, conveys fluid back to the aerator and the other leads to the cannula into the hind limb. Since the greater portion of the output of the syringe is returned to the aerator the turnover of fluid therein is very rapid and stirring of the red blood cells is

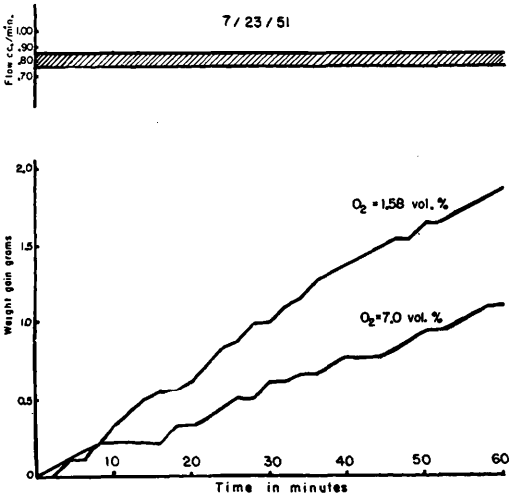

FIG. 2. Representative data relating weight gain, blood flow and oxygen content of perfusion fluid as a function of time.

13. Hyman, C., *Am. J. Physiol.*, 1944, v142, 671.

14. Clark, L. C., Gupta, V. B., and Gollan, F., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 268.

15. Ramsay, W. N. M., *Biochem. J.*, 1946, v40, 286.

TABLE I. Summary of Data Relating Edema Formation with Oxygen Content and Flow Rate of the Perfusion Fluid.

Time, min	No. of expts.	Perfusion fluid, O_2 vol %	Avg mean press., mm Hg	Avg flow, cc/min	Avg wt gain, g/min/100 g
0-20	6	.88-2.60	41	.54	.142
	5	5.50-9.40	67	.60	.100
21-40	6	.88-2.60	31	.52	.086
	5	5.50-9.40	60	.60	.046
41-60	6	.88-2.60	32	.52	.093
	5	5.50-9.40	67	.60	.037

more effectively achieved. A screw clamp on the backflow tube could be adjusted to permit any desired flow through the cannula. A mercury manometer is placed in the perfusion system at the attachment of the cannula for the continuous recording of mean perfusion pressure on a slowly moving kymograph. The isolated preparation is placed on an aluminum pan suspended from a sensitive spring. At the junction of the spring and the pan one end of a heart lever is inserted so that a downward displacement of the weight pan is magnified 14 times by the mechanical advantage of the lever. The weight pan is allowed to move freely in a vertical plane along a guide wire. The pan is tilted so venous blood from the limb falls off the pan causing an instantaneous drop in weight and giving the weight tracing a serrated appearance. The number of drops falling off per minute is used as a measure of flow. At the beginning of each experiment the spring is calibrated by adding one gram weights and noting the vertical displacement of the lever, usually about 6 mm per gram. The slope of edema formation during a given period of time is calculated by the least squares method, expressing the slope in g/min./100 g of tissue.

Male albino rats weighing 250 to 350 g are anesthetized with ether and the right hind leg is cannulated with a flexible, fine polyethylene cannula in the iliac artery at the bifurcation of the aorta. The limb is dissected from the body at the hip, tying off all of the large vessels that are cut except the vena cava through which most of the outflowing blood passes. The perfusion is started immediately after the cannulation is complete to avoid stagnation of blood in the leg. The isolated limb is weighed on a beam balance before and after perfusion. An infra red lamp placed about 1½ feet from the leg prevents cooling of the exposed tissues. The perfusion fluids used consist of 20% washed dog red blood cells in 0.33% ash free gelatin in Kreb Ringer solution. The low colloidal content of these perfusion fluids is necessary to obtain a steady rate of edema formation within an experimental period of one to two hours. The pH of the fluids is adjusted to 7.4 with Na_2HPO_4 and is measured with a Beckmann glass electrode pH meter. Hemoglobin is determined by the Sahli method and the oxygen content by the Roughton and Scholander microgasometric syringe analyzer method(16).

Results. In the early experiments perfusions were carried out with hypoxic or with oxygenated blood at a constant mean perfusion pressure of 90 mm Hg. Under these conditions the flow during the hypoxemic perfusion was several times greater than during oxygenated perfusion in the same leg, thereby tending to compensate for the reduced oxygen content of the nitrogenated blood. Therefore, it was desirable to maintain flow constant in order to make manifest any changes due to hypoxemia *per se*. Consequently, in the following experiments the standard or reference flow was determined for each hind limb by perfusing it with oxygenated blood at 90 mm Hg mean perfusion pressure and noting the flow under these conditions. During the subsequent experimental period of one hour the flow was maintained at that reference level. Eleven perfusions were made; 6 with hypoxic blood (0.88 to 2.60 vol. %) and 5 with oxygenated blood (5.50 to 9.40 vol. %). Usually one hind leg was perfused with hypoxic blood

16. Roughton, F. J. W., and Scholander, P. F., *J. Biol. Chem.*, 1943, v148, 541.

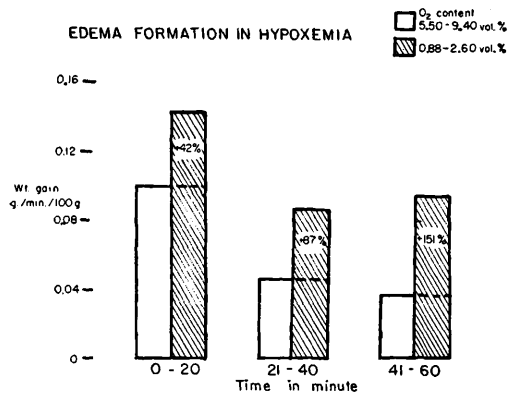

FIG. 3. Description in text.

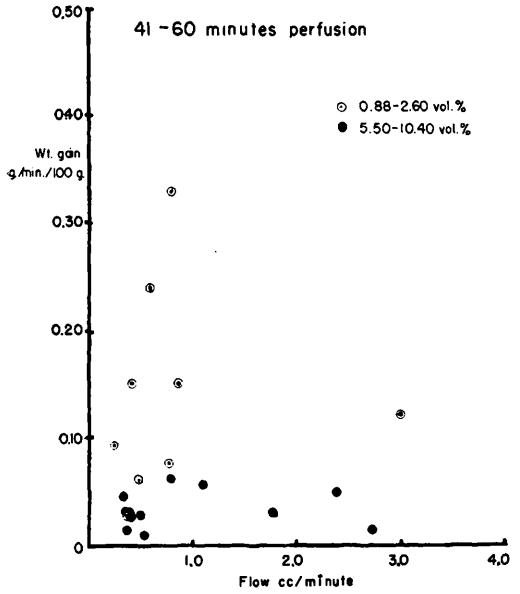

FIG. 4. Relationship between edema formation and flow.

and another with an oxygenated portion of the same blood for one hour. The slope of edema formation was calculated for the three consecutive 20-minute periods in each experiment. The protocols of 2 typical perfusions are shown in Fig. 2. The hypoxemic leg (1.58 vol. %) gained 1.87 g in one hour and the leg with oxygenated blood (7 vol. %) gained 1.1 g. In both cases the flow was maintained within a constant range of 0.75 to 0.85 cc/min. The results of 11 such experiments are summarized in Table I.

When the final column of rate of edema formation in g/min./100 g of tissue is plotted on a bar graph (Fig. 3) the effect of time and of oxygen content is more readily seen. With adequate oxygenation (solid bars) the edema rate is greatest in the first 20 minutes of perfusion and falls progressively during the rest of the hour. During hypoxemia, however, (striped column) the edema rate is 42% greater than the oxygenated values in the first 20 minutes, 87% greater in the next 20 minutes, and 151% higher from 41 to 60 minutes. This indicates that as anoxia of the tissues progresses, edema formation becomes more pronounced.

In Fig. 4 the blood flow is plotted against edema rate in 21 experiments in the last 20-minute period when the hypoxic changes were the most severe. During hypoxemia (dotted circles) the edema rate was approximately proportional to flow, whereas when the blood was fully oxygenated (solid circles) the edema rate was not altered significantly by flow.

Discussion. This preliminary work indicates that oxygen content above 5 vol. % plays no significant role in altering the rate of edema formation in the isolated rat hind limb perfused with blood low in colloidal content. At lower oxygen values an increased porosity of the capillaries is suggested since with increasing flow the rate of edema formation also increases. This was not demonstrated in the adequately oxygenated preparations.

We are in agreement with the conclusions of Henry(9) and Maurer(2,3) who state that the blood oxygen must fall below 4 to 6 vol. % or 15 to 20% saturation before a permeability change is noted. It would appear that the oxygen level at which a change in capillary function is detected is incompatible with life so that generalized hypoxemia can hardly be considered as an etiological factor in edema formation in chronic pathological states.

Summary. (1) A pulsatile perfusion system is described in which temperature, blood flow and mean pressure are controlled, and in which red blood cell solutions may be satisfactorily perfused through isolated limbs and organs. (2) In hind limbs perfused with blood of low colloidal content, 5.5 to 9.4 vol. % oxygen content were adequate in main-

taining capillary function since a minimal rate of edema formation was observed which did not vary appreciably with flow. (3) Hind limbs perfused with 0.88 to 2.60 vol. % oxygen exhibited augmented edema formation which increased with increased flow. The critical hypoxemia level affecting capillary permeability is believed to lie between 2.6 and 5.5 vol. % oxygen content for the experimental conditions described.

Received October 15, 1951. P.S.E.B.M., 1951, v78.

Utilization of Ammonium Nitrogen for Growth and Toxin Production by a Strain of *Corynebacterium diphtheriae*. (19143)

RUTH M. DREW. (Introduced by W. M. Hale.)

From the Division of Bacteriology and Virology, Brookhaven National Laboratory, Upton, L. I., N. Y.

In general it has been believed that *Corynebacterium diphtheriae* utilized only nitrogen from organic materials. However, it has been shown by Drew and Mueller(1) that the Toronto strain of Park-Williams no. 8 readily utilizes ammonium nitrogen in addition to that from organic substances. On chemically defined medium it was found that in the presence of optimal concentrations of amino acids growth was poor without added ammonium sulfate or ammonium chloride. The fact that glutamic acid was an essential amino acid for the test strain and that the addition of ammonium salt resulted in greater growth suggested that perhaps ammonia was being utilized to convert glutamic acid to glutamine. Hac, Snell and Williams(2) and McIlwain(3) have demonstrated that certain microorganisms readily perform the conversion of glutamic acid to glutamine when ammonia is available. The possible role of ammonia in the conversion of glutamic acid to glutamine by the Toronto strain has therefore been investigated.

Quantitative studies involving the utilization of ammonium nitrogen for the growth of the Toronto strain of Park-Williams no. 8 are to be reported. It will be shown that glutamine in high concentrations can substitute for glutamic acid and ammonium sulfate for the growth of the test strain. Glutamine plus ammonium ion has a greater additive effect than glutamine alone, indicating that ammonia is used in some other way, or in addition to glutamine synthesis.

Material and methods. A chemically defined medium known to support excellent growth of the Toronto strain and the production of high titer toxin was employed. The composition and preparation of the medium as well as the methods for cultivation are the same as previously described by Drew and Mueller(1). Total bacterial growth was estimated on the washed cells according to the Pregl micro-Kjeldahl method as adapted by Mueller(4) and expressed as "mg of bacterial nitrogen".

Experimental. Several experiments were carried out in which the growth curves produced by glutamic acid (sodium glutamate) were compared with and without ammonium sulfate added to the basal medium, and glutamine was tested as a substitute for sodium glutamate and ammonium salt. It was found that the basal medium to which sodium glutamate was added did not support good growth of the test strain and that sodium glutamate metabolism was accompanied by an accumulation of alkali (decarboxylation with the formation of (Na_2CO_3 and $NaHCO_3$)). Optimum growth took place on medium containing sodium glutamate plus ammonium sulfate, and it was observed that the presence of the

1. Drew, R. M., and Mueller, J. H., *J. Bact.*, 1951, v62, 549.

2. Hac, L. R., Snell, E. E., and Williams, R. J., *J. Biol. Chem.*, 1945, v159, 273.

3. McIlwain, H., *J. General Microbiol.*, 1948, v2, 186.

4. Mueller, J. H., *J. Bact.*, 1935, v29, 383.