solved in the 0.02 M phosphate buffer pH 7.3, diluted to 0.1 mg protein/ml and pH was adjusted to 7.5. It is believed that the 4-5 fold increase in purification shown in Fig. 2, is due more to the very dilute solution of protein fractionated rather than change in pH. As in the first ethanol fractionation over 100% recovery of the enzyme being fractionated was obtained. This increase could not be attributed to increased stability of the enzyme at assay pH of 7.0, because the enzyme was stable at this pH for at least 3 weeks after the second ethanol fractionation. It seems more likely to be due to removal of inhibitors during the purification procedure.

An apparent over-all recovery of 45% was obtained by this procedure and the enzyme was purified 1000-fold. However, it is obvious from the data that removal of inhibitors during purification may account for part of this high yield. In small scale experiments, it appeared that if the second ethanol was conducted at pH 7.0 instead of 6.8 even higher yields could be obtained.

Rogers(6,9) does not mention the loss of *Cl. perfringens* hyaluronidase below pH 7.0. In fact, we may assume that this did not occur with his strain since it was absorbed on aluminum oxide at pH 4.6 (a pH which would have caused immediate destruction of hyaluronidase preparations obtained in these laboratories). The marked difference in stability of these two hyaluronidase preparations cannot be explained at present. Conditions of growth, as well as strains, permit many possibilities. However, it is suggested that two hyaluronidase disaccharases may be present in these organisms. Consequently, the two laboratories may not have been dealing with the same enzyme.

Summary. A procedure has been detailed for a 1000-fold purification of a bacterial hyaluronidase from *Cl. perfringens,* strain 2278. Initial crude fractionation with ammonium sulphate followed by three ethanol fractionations at pH 9.0, 6.8 and 7.5 respectively have yielded hyaluronidase fractions containing 200000 TRU/mg N with recovery of 45% of the crude enzyme.

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Production of Impending Hepatic Coma by a Carbonic Anhydrase Inhibitor, Diamox.*† (22159)

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Impending hepatic coma, characterized by confusion and the typical "flapping" tremor, has been described previously in alcoholics with cirrhosis given oral nitrogenous substances—ammonium cation exchange resins, ammonium salts, urea, and increments in dietary protein(1-3). The onset of this syndrome is here described in similar patients following oral administration of a carbonic anhydrase

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TABLE I. Clinical and Laboratory Data of 12 Alcoholics with Cirrhosis Given an Oral Carbonic Anhydrase Inhibitor, Diamox.

Patient, age, sex	BSP†/Ser. bil. (mg/100 ml)	Diamox–Dose, frequency,‡ duration	KCl daily dose (g)	Mental state during Diamox‡	"Flapping" tremor during Diamox	Avg periph. vein blood NH_3-N** (γ/100 ml) Before§	During‖
		Group I (Developed impending coma during Diamox)					
		mg Days					
J.H.,* 49, ♀	35/ 1.2	500 A 3	0	C	Appeared	—	—
		" A 13	6	C	"	51 (2)	138 (8)
		" D 6	6	C	"	81 (2)	105 (4)
			Started 3rd day				
J.M.,*¶ 32, ♂	36/ 4.5	" A 3	0	C	"	—	—
J.S.,¶ 44, ♂	—/ 8.0	" D 7	3	C	More severe	65 (3)	116 (4)
J.T.,¶ 50, ♂	33/ 3.5	1000 D 5	0	C	0	55 (3)	50 (3)
		Group II (No impending coma)					
H.D.,* 40, ♀	48/10	500 A 27	0	Dr	0	63 (4)	87 (17)
	16/ 1.2	" D 3	0	N	0	78 (1)	69 (1)
E.F.,* 37, ♀	—/ 4.3	" A 27	0	N	0	62 (3)	87 (19)
J.Mc.,* 45, ♂	25/ 1.3	" D 7	0	N	0	74 (2)	89 (5)
G.A., 52, ♂	—/15.6	" D 6	0	N	0	85 (4)	92 (4)
F.J., 62, ♂	31/ 2.0	" D 8	0	N	0	59 (3)	66 (4)
C.O'D., 49, ♀	29/	500 D 7	0	Dr	0	73 (4)	88 (6)
		Then 1000 D 2					
G.D., 42, ♀	51/ 4.7	1000 D 7	0	N	0	64 (3)	73 (4)
M.B., 47, ♀	—/ 6.9	" D 5	0	Dr	0	48 (2)	55 (4)

* Diagnosis of Laennec's cirrhosis confirmed by postmortem or liver biopsy.
† Bromsulfalein: % retention in 45 min.
‡ A = Alternate days; D = Daily; C = Confused; Dr = Drowsy; N = Normal.
§ Before Diamox administration.
‖ During " "
¶ History of previous mental symptoms associated with liver disease.
** Numbers in parentheses represent the number of determinations.

inhibitor, acetazolamide (Diamox)‡(4,5). This drug, as well as sulfanilamide, has been employed in the treatment of cardiac failure because it may cause a sodium and water diuresis(6,7).

Materials and methods. From January, 1954, through January, 1955, on the Thorndike Ward, 12 alcoholic patients with cirrhosis received 15 courses of Diamox therapy (Table I). One patient received 3 courses, another 2. Six patients were men and 6 women, ranging from 32 to 62 years of age. Diagnosis of cirrhosis was made by history of prolonged alcohol and poor food intake coupled with physical and laboratory evidence consistent with severe long-standing liver disease. Six of the 12 patients had mild hemolytic anemias thought to be consistent with liver disease(8) and 3 had peripheral neuritis. Rheumatic heart disease, treated "beri-beri" heart disease, and psoriasis were diagnosed in one instance each. Diagnosis of cirrhosis was confirmed by postmortem examination in patients J. M. and J. H., who died several months later, and by liver biopsy in 3 other patients. One of the latter, J. Mc., also had peritoneal tuberculosis when biopsy was performed 3 months after Diamox. All patients had fluid accumulation and received weighed diets containing less than 270 mg of sodium and from 66 to 75 g of protein daily. An oral vitamin supplement Vi Magna Granules,§ containing vit. A, B complex, C, and D, was added daily. Stools were examined frequently for occult blood. Blood was drawn in the fasting state or 2 to 3 hours postprandially in the morning. Hematocrits, serum carbon dioxide contents, and concentrations

‡ Provided through the courtesy of Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.

§ Provided through the courtesy of J. M. Ruegseggar of Lederle Laboratories Division of American Cyanamid Co., Pearl River, N. Y.

of nonprotein nitrogen, sodium, potassium, and chloride, were determined using methods previously described(9). Serum bilirubin, 45-minute bromsulphalein retention, cephalin flocculation, thymol turbidity and flocculation were also determined using standard methods. One ml of peripheral venous blood drawn in a heparinized syringe was immediately injected into the alkaline outer chambers of each of 2 Conway units and analyzed for "ammonia" nitrogen (NH_3-N)‖ using McDermott and Adams' modification of the Conway technic(10). Our values were lower than those of McDermott *et al.*, but recoveries from standard solutions were good. The normal range was from 29 to 86 mg NH_3-N per 100 ml (16 patients, 19 determinations). Before Diamox 71 control determinations in 12 patients with cirrhosis ranged from 28 to 114 μg per 100 ml with only 2 determinations exceeding 100. Eight patients with spontaneous impending hepatic coma, not included here, had concentrations (21 determinations) ranging from 35 to 246 μg per 100 ml and 6 of these were below 114. Blood NH_3-N determinations during Diamox administration were made on the morning following initial dose and most mornings thereafter up to and including the morning the drug was discontinued. Concentrations presented (Table) represent averages; figures in parentheses represent the number of determinations from which averages were calculated. After blood NH_3-N determinations were made, Diamox was given in amounts of from 500 mg on alternate days to 1000 mg daily from 3 to 27 days. Potassium chloride was given in daily oral quantities of 3 g to one patient (J.S.) and 6 g to another (J.H.); the latter received it before and during one course of Diamox and also during part of another (Table).

Confusion and the typical "flapping" tremor(11) were not present before Diamox except in J. S., who had a slight tremor.

Results. Two groups were established (Table): Group I, 4 patients who developed confusion with or without tremor, and Group II, the remaining 8, none of whom developed this syndrome. All in Group I had chronic severe cirrhosis and 3 had previously had impending hepatic coma. Of the 8 in Group II, 5 had cirrhosis of a severity comparable to those in Group I, but none had had impending coma.

Beginning within 2 to 48 hours of the initial dose, 4 patients developed confusion, during 6 periods of Diamox administration, 2 after only 500 mg. Tremor remained absent during one period, became accentuated in one, and appeared in 4. Confusion became marked in 2 patients (J.H. and J.M.) and remained mild in the other 2. Except in J.M. it did not progress after the first 48 hours and improved in J.S. and J.T. despite continuation of Diamox. The tremor usually persisted longer.

During Diamox administration average venous blood NH_3-N concentrations rose above those of the highest control determination (114 μg per 100 ml) during 2 of 4 periods. During Diamox, average NH_3-N concentrations and increases in these 2 instances exceeded all control averages in Group II at the 5% level or less. Concentrations fluctuated from day to day and were usually higher in the first 72 hours, falling later despite continuation of Diamox. Average serum potassium concentrations decreased during all 6 periods, during 4, 0.4 mEq or more. Potassium chloride, administered during 3 periods, failed to prevent or alleviate impending coma although average serum potassium concentrations remained above 4 mEq per l in patients who received it.

In Group II, confusion and tremor were absent, although drowsiness was present in 3 of 8 patients during Diamox administration. Average blood NH_3-N concentrations increased during all except one period. Day to day values, highest in the first 72 hours, fluctuated, sometimes reaching abnormally high levels. In one of 7 periods the average serum potassium concentration decreased more than 0.4 mEq per l, and during another a low value (2.7 mEq per ml) was found.

‖ Ammonium ion is either present in shed blood or formed from nitrogenous substances in blood shortly after shedding. Volatile blood base, liberated by adding strong alkali, is probably all or nearly all ammonia.

The 2 groups showed no significant difference in serum carbon dioxide contents or concentrations of sodium and chloride before or after Diamox. Serum sodium concentrations rose slightly during 6 and remained constant during 8 periods of Diamox therapy; serum chloride concentrations rose during 9 periods, remained the same during 3, and dropped slightly during one. Serum carbon dioxide contents decreased during 11 periods and remained the same in 3. Acidosis (a rise in serum chloride concentration and fall in carbon dioxide content) occurred even when the smallest doses of Diamox were given.

Discussion. Clinical signs and symptoms which may occur after Diamox administration are well documented by Maren *et al.*(12). Noted in patients without liver disease have been paresthesias, drowsiness, and deep sleep. Confusion was not reported and we did not observe it in 12 patients given single 500 mg oral doses of Diamox.

In cirrhosis of the liver, however, bizarre changes of mood, hallucinations, loss of memory, confusion, and occasional psychotic episodes were first described by Eisenmenger (13) to occur following Diamox administration. Confusion also appeared here, but was, in addition, often associated with the typical "flapping" tremor which occurs in impending hepatic coma.

Impending hepatic coma, characterized by confusion and "flapping" tremor in patients with liver disease, is often but not always associated with increased venous blood NH_3-N concentrations(14). Two of our 3 patients with confusion and tremor had significant increases and elevated concentrations of blood NH_3-N after Diamox. The nitrogen contained in Diamox was not the source of increased blood NH_3-N because in cirrhosis this drug is nearly all excreted in the urine(13). The pathogenesis of impending hepatic coma after Diamox is different, then, from that induced by nitrogenous substances thought to liberate ammonium in the gastrointestinal tract.

Diamox may have a direct effect on brain metabolism because parasthesias occurring in the first 8 hours after Diamox improved as the plasma concentrations of Diamox could be predicted to be falling and because administration of sulfanilamide, another carbonic anhydrase inhibitor, produced confusion in a large percentage of patients without liver disease(12). The mechanism of action is unknown, although sulfanilamide interferes with maintenance of potassium gradients in guinea pig brain slices(15). Decreases in serum potassium concentrations were more marked here in the group developing impending coma, but mental symptoms occurred at normal serum potassium concentrations and potassium salts produced no improvement. Possibly, the brain of patients with severe hepatic disease is more sensitive to Diamox because only these patients developed confusion in our hands.

Conclusions. 1. The carbonic anhydrase inhibitor, Diamox, can produce impending hepatic coma in susceptible alcoholics with cirrhosis. 2. This type of induced impending coma may be associated with a rise in femoral or antecubital vein blood ammonia nitrogen concentrations to levels above those seen in mentally clear patients with cirrhosis. 3. The nitrogen contained in Diamox is not a source of the elevated blood ammonia nitrogen concentrations.

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Residual Organ Blood Volume of Cattle, Sheep and Swine.* (22160)

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In quantitative studies involving investigations of tissue distribution of blood-borne substances a correction for the residual organ blood may be essential. This is of special importance in short time radioisotope distribution experiments where retained tissue blood may be sufficient to contribute significantly to the total activity recovered. Specific studies based upon whole tissue extractions measure not only the substance in the cells of the tissues, but also that existing in the blood which is trapped within these tissues at the time of sampling. Therefore, in tracing the distribution of an ingested or injected substance by extraction from various organs, it is necessary to know the proportion of the material that is from the tissue and that which is from the blood itself. When the relative proportions of blood and tissue are known, precise quantitative studies on movement and tissue composition may be used to substantiate microscopic examinations and organ weight changes. The total organ weights for farm animals have been reported by several investigators(1-3), and procedures and values for blood volume of cattle, sheep and swine are in the literature(4-6). Lewis *et al.*(7) have reviewed procedures and reported the total organ blood volume measurements for the rat. Other investigators have made *in vitro* and *in vivo* measurements of total organ blood volumes in laboratory animals(8,9) but there were no data available from the literature directly related to the residual blood present in organs and tissues of farm animals slaughtered by the conventional procedures.

This report is concerned with the residual blood values as related to age and species for the various organs and tissues of farm animals routinely sacrificed and bled out during current nutritional investigations.

Material and methods. The cattle, sheep and swine used in these studies were normal, healthy individual animals maintained under general farm management conditions. Total red blood cell volumes were obtained by the injection of P^{32} labeled homologous erythrocytes as previously described(4). The organ blood values were procured as follows: (a) blood samples were drawn from the animal; (b) whole blood was incubated with P^{32}; (c) red blood cells were washed sufficiently to remove any radioactivity not in the cells and (d) the cells were reconstituted to the original volume with the animal's own plasma. (e) A known volume of suspended labeled cells was re-injected into the immobilized animal(10) and after mixing had occurred, blood samples were taken from the jugular vein of the animal, usually at 5 and at 15 minute intervals for radioactivity measurement and blood volume determinations. (f) The animal was then sacrificed by a blow

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