TABLE I.
Effect of Egg Yolk upon Infection of Mice with Tubercle Bacilli (H37) via Intraperitoneal Route.

Culture H37	Egg yolk suspension	Wt. at weekly intervals after infection				No. dead/ Total inoculated
		Initial	1 wk	2 wk	3 wk	
cc	cc	g	g	g	g	
0.25	0	20.0	20.2	22.6	24.2	0/6
0.25	0.25	20.5	20.7	18.0	Dead	6/6

TABLE II.
Comparison of H37 (Virulent) and H37Ra (Avirulent) Strains Inoculated Via Intravenous Route.

Culture		Egg yolk suspension	Wt at weekly intervals after inoculation					No. dead/ Total inoculated
			Initial	1 wk	2 wk	3 wk	4 wk	
	cc	cc	g	g	g	g	g	
H37	0.01	0	18.3	21.3	22.2	19.7	19.2	5/6
H37Ra	0.2	0	18.3	21.6	24.2	25.9	26.7	0/6
H37Ra	0.2	0.1	18.3	20.9	23.3	24.6	25.3	0/6

differences in susceptibility among the different strains of mice. In general, albino mice derived from the so-called Swiss strain are somewhat more resistant than the white mice of the Rockefeller Institute strain. On the contrary, a number of other strains such as C57 black, dba, and wild mice *(Mus musculis domesticus)* are markedly more susceptible. The C_3H strain is intermediate between the latter group and the Rockefeller Institute strain.

Albino Swiss mice die within 3 weeks following intravenous injection of 0.05 cc of H37 culture; they survive and exhibit only limited pulmonary lesions when infected with smaller doses. On the other hand, 0.003 cc of culture is sufficient to cause death of dba mice within 3 weeks; moreover, animals of this strain, sacrificed 4-5 weeks after intravenous injection of 0.00003 (corresponding to 0.000005 mg dry weight becilli), show extensive pulmonary lesions often involving whole lobes.

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Alpha Naphthylthiourea (ANTU) in Dogs: Electrophoretic and Cholesterol Studies on Blood Plasma and Pleural Effusion.*

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Richter[1] recently described a new rat poison, alpha naphthylthiourea (ANTU), which exerts its toxic effect within a few hours by increasing the permeability of the pulmonary and pleural capillaries. In the dog and rat, the pulmonary edema and pleural effusion cause death. An analysis of the electrophoretic patterns of the serum and effusion fluid in animals poisoned with ANTU provides an approach to the problem of diffusion of proteins across damaged capillary walls.

* This work was done under contract with the Medical Division of the Chemical Warfare Service.

[1] Richter, C. P., *J. Am. Med. Assn.*, 1945, **129**, 927.

TABLE I.
Analyses of the Electrophoretic Patterns of Serum and Pleural Effusion After Lethal Doses of ANTU.

Hr after ingestion	% area				Mg N %			
	Albumin	α_1 α_2	α_3	β_1 β_2 γ	Albumin	α_1 α_2	α_3	β_1 β_2 γ
75 mg/kg ANTU in gelatin capsule by mouth.								
Dog 1: Wt—27.3 kg.								
Serum.								
Control	51	14	35		492	135	338	
4	49	17	34		480	166	333	
6	48	16	36		446	149	335	
10	48	21	32		385	168	257	
12	45	24	32		373	199	265	
13½	47	18	35		307	118	229	
15	43	22	34		279	143	221	
Pleural Effusion.								
15	54	12	34		388	86	244	
Dog 2: Wt—20.6 kg.								
Serum.								
Control	47	16	30	5	540	184	345	58
7	46	12	34	6	520	136	384	68
14	45	13	35	6	476	138	371	64
15	46	15	33	6	527	172	378	69
16	47	13	34	7	446	124	323	67
17	52	10	31	7	505	97	300	68
Pleural Effusion.								
15	46	12	36	5	324	85	254	35
16	47	15	38		329	105	266	
17	53	10	37		366	69	255	
Dog 3: Wt—20 kg.								
700 mg ANTU intravenously injected in a 6% acacia-saline mixture.								
Hr after injection			Serum.					
0	40	19	33	8	440	209	363	88
6	40	18	33	9	388	175	320	87
7	41	18	35	6	400	176	341	58
7½	37	23	31	9	396	246	332	96
Pleural Effusion.								
6	49	13	38		318	85	247	
7½	49	10	34	7	360	74	250	52
Dog 4: Wt—15.9 kg.								
560 mg ANTU injected intravenously.								
Serum.								
0	47	14	32	7	545	162	371	81
7	39	19	33	9	392	177	307	84
Pleural Effusion.								
7	53	12	28	7	379	86	200	50

Richter also observed that if a rat ingests a sublethal dose of ANTU, its tolerance for larger amounts develops rapidly and persists for a comparatively long period. The thiourea portion of the ANTU molecule probably affects the thyroid metabolism of these animals. A number of investigators have found that thiourea and its derivatives depress the functional activity of the thyroid gland by interfering with the synthesis of thyroxine and diiodotyrosine.[2,3] In man, thiourea administration causes a rise of blood cholesterol[4] which may be expected in hypothyroidism. Moore, Levin, and Smelser[5] showed than an α-globulin component of rat sera increases after thyroidectomy or after thiouracil feeding.

This report deals with (a) the protein and cholesterol concentrations of the serum and pleural effusion in dogs dying after oral or intravenous administration of ANTU; and

[2] Astwood, E. B., *J. Am. Med. Assn.*, 1943, **122**, 78.

[3] Chen, K. K., *Ann. Rev. Physiol.*, 1945, **7**, 677.

[4] Jennings, L. M., Mawson, C. A., and Tindall, W. J., *Lancet*, 1944, **2**, 91.

[5] Moore, D. H., Levin, L., and Smelser, G. K., *J. Biol. Chem.*, 1945, **157**, 723.

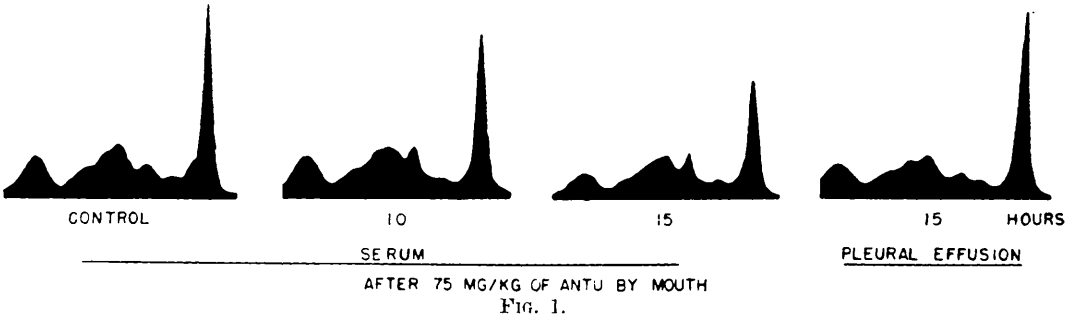

FIG. 1.

Electrophoretic patterns for the serum and pleural effusion of Dog 1 after the administration of ANTU.

(b) the protein and cholesterol concentrations of the serum of dogs which had developed a tolerance to large amounts of this drug.

Methods. Electrophoretic analyses were done in the Tiselius apparatus, using the scanning method of Longsworth[6] and single section cell of 11.0 ml capacity. The serum was diluted 1.5 times with barbiturate buffer, ionic strength 0.1, at pH 8.6. Electrophoresis proceeded for 120 minutes at 15 m. amp. and 200 volts, with a potential gradient of 6.0 volt per cm at 2.0°C.

Cholesterol was determined on plasma by the Schoenheimer-Sperry procedure[7] with the aid of an Evelyn photoelectric colorimeter with filter No. 660; known amounts of recrystallized cholesterol were used for calibration. The micro-Kjeldahl method was used for total and nonprotein nitrogen.

Results. Protein Distribution. The electrophoretic analyses of 4 dogs given ANTU orally and intravenously are presented in Table I. Owing to the indistinct nature of the electrophoretic patterns of these sera, the proteins are grouped as (1) albumin and α_1, (2) $\alpha_2 + \alpha_3$, (3) $\beta_1 + \beta_2$, and (4) γ globulins, or a combination of (3) and (4). In group (1), α_1 globulin represents a small proportion of the protein. The data are expressed as percent of total protein and as milligrams percent of nitrogen.

Acute Poisoning. Satisfactory sampling of blood was attained in 2 of 6 dogs which were given ANTU in gelatin capsules by mouth, and 2 of 4 dogs intravenously injected with ANTU.[8] In all animals the pulmonary edema was very marked and responsible for their death. Varying amounts of blood-tinged pleural effusion were seen in 9 of the 10 dogs. Usually some respiratory distress was exhibited several hours before death, and great difficulty during the last hour. It was impossible to obtain sufficient serum (10-15 ml) from 6 dogs despite the fact that 100 to 200 ml of blood were obtained when the animal was sacrificed by exsanguination. The blood, of tarry consistency when freshly drawn, was allowed to stand for several hours and was then centrifuged at high speeds. The clot was not firm and did not retract over a period of at least 5 hours. Standing for longer periods yielded a serum which was markedly hemolyzed.

The essential details concerning the 4 satisfactory experimental animals are discussed.

Dog 1. (Table I). Blood was drawn at intervals and the dog sacrificed 15 hours after oral administration of ANTU. The serum albumin concentration decreased markedly and the nitrogen concentration of the $\beta_1 + \beta_2 + \gamma$ globulin group was also lowered.

In the effusion fluid, the albumin concentration was greater than that of the serum at the time of death; the $\alpha_2 + \alpha_3$ group was smaller. The electrophoretic patterns of serum and pleural effusion (Fig. 1) show a striking similarity.

Dog 2. (Table I). The chest was tapped at intervals and the first sample of effusion

[6] Longsworth, L. G., *J. Am. Chem. Soc.*, 1939, **61**, 529.

[7] Schonheimer, R., and Sperry, W. M., *J. Biol. Chem.*, 1934, **106**, 745.

[8] Drinker, C. K., *Pulmonary Edema and Inflammation*, Harvard University Press, 1945, 39-43.

TABLE II.
Electrophoretic Analyses of Sera of Dogs 5 and 6 After Multiple Oral Doses of ANTU. Grams Protein in 100 ml Serum.

Dog No.	Experimental day	ANTU mg/kg	% distribution			Mg N in 100 ml serum		
			Alb.	$\alpha_1 + \alpha_2$	$\beta + \gamma$	Alb.	$\alpha_1 + \alpha_2$	$\beta + \gamma$
5	1	10	51	14	35	465	130	326
	3	10						
	4		47	18	35	454	174	331
	6	15						
	8	25	46	18	36	457	172	352
	10	40	46	21	33	444	206	321
	13	60	42	22	36	411	219	358
	15	75	40	24	36	387	235	355
	17	75	42	23	35	400	214	337
	20	150	39	22	39	390	224	392
	22		48	22	30	515	232	323
	27		46	22	32	470	230	331
	31		44	21	35	403	198	318
6	1	10	56	16	28	385	110	195
	3	10						
	4		49	25	26	385	198	201
	6	15						
	8	25	48	20	32	395	164	257
	10	40	47	21	32	380	168	257
	13	60	49	20	31	406	161	254
	15	75	45	21	34	374	174	278
	17	75	47	21	32	376	172	249
	20	150	48	19	32	416	164	270
	24		49	20	31	388	155	240
	27		50	24	26	427	208	217
	41		50	21	29	424	180	249

fluid obtained 15 hours after oral administration. The percentage composition of the serum proteins remained constant throughout the experiment, but the protein concentrations decreased. Appreciable decreases in the milligrams percent of nitrogen occurred in all groups except the γ globulin.

The percentage distribution of the protein groups was almost identical for the serum and effusion fluid at the respective periods. The total nitrogen concentration of the effusion was less than that of the serum, and therefore the content of the various protein groups was proportionately smaller.

Dog 3. (Table I). ANTU was injected intravenously and the chest was tapped hourly. The first effusion fluid was obtained 6 hours after injection at which time blood was also drawn. The dog showed signs of distress shortly afterwards and died 7½ hours after injection. Respiration ceased before the animal could be exsanguinated. Blood was drawn from the heart and approximately 200 ml of effusion fluid were obtained.

The changes in the concentration of the serum fractions were not pronounced. The percentage of the albumin fraction in the pleural effusion was greater than in the serum and the milligram percentage of nitrogen was slightly lower. The nitrogen concentrations of the globulin groups were much lower than those of the serum.

Dog 4. (Table I). This animal was exsanguinated 7 hours after intravenous injection of ANTU. The serum albumin concentration was decreased markedly at death and the globulin groups were only slightly affected. The albumin nitrogen concentration of the effusion fluid was about the same and the concentrations for the globulins were less than those of the serum.

Chronic Poisoning. Two dogs, fed increasingly larger amounts of ANTU in a gelatin capsule, developed a tolerance for doses ordinarily lethal to control animals. The perti-

TABLE III.
Plasma and Pleural Effusion Cholesterol.
Dog given 75 mg/kg of ANTU by mouth.

Hr after ANTU	Total cholesterol, mg %
Plasma.	
Control	95
4	97
6	107
10	91
12	87
13½	79
15	72
Pleural Effusion.	
15	67

nent data for drug administration and for protein distribution in the serum are shown in Table II. There is no typical change in the protein distribution, except a moderate increase in α globulin concentration which can be correlated with the development of tolerance for ANTU.

Plasma Cholesterol. The data for the plasma and effusion cholesterol concentrations after acute poisoning (Table III) indicate a definite decrease in the plasma a few hours before death. The cholesterol concentrations of the plasma and pleural effusion were the same.

Chronic poisoning in 2 dogs was accompanied by a marked increase in plasma cholesterol concentration which probably reflected the "chemical thyroidectomy" caused by ANTU (Table IV). An immediate increase in cholesterol was seen after the first small doses. The values continued to increase while the ANTU was administered. After its discontinuance, the plasma cholesterol concentration decreased rapidly.

TABLE IV.
Effect of Chronic ANTU Feeding on Plasma Cholesterol of Dogs.

Day of exper.	ANTU administered mg/kg	Plasma cholesterol mg %	
		Dog 5	Dog 6
1	10	95	118
3	10		
4		153	165
6	15		
8	25	166	176
10	40	194	195
13	60	235	191
15	75	255	215
17	75	287	228
20	150	254	235
22		215	235
24		216	178
27		150	127
31		135	111
41		98	87

Discussion. In ANTU poisoning, the decrease in the concentration of the protein fractions, particularly in albumin, is apparently a result of the loss of plasma into the lungs and effusion. In the 2 experiments in which the proteins of the pleural effusion were measured before and at death, the albumin concentration increased with time; the data for the globulins are insufficient for drawing any conclusion.

Results of a study of plasma and effusion proteins in a variety of diseases by Luetscher[9] indicate that the "albumin is present in the same or higher proportion in the effusion, as compared with plasma." Cohn[10] believes that the factor determining diffusion of a protein across a permeable membrane "should be an inverse function of the length of its molecule." This idea is borne out by the data presented by Luetscher,[9] and by the present experiments.

The effect of ANTU on cholesterol metabolism is striking. The increases noted in chronic experiments are probably associated with the thiourea portion of the molecule and its action on the thyroid. Direct observations on the thyroids were not made.

Summary and Conclusion. Oral and intravenous administration of lethal doses of alpha naphthylthiourea (ANTU) to 4 dogs caused death by affecting the permeability of pulmonary and pleural capillaries, which resulted in marked pulmonary edema and pleural effusion. Electrophoretic and nitrogen determinations of the sera showed consistent decreases in the concentration of albumin several hours before death. The proteins of the pleural effusion showed a higher albumin : globulin ratio than those of the serum. The total plasma cholesterol concentration decreased shortly before death and

[9] Luetscher, J. A., Jr., *J. Clin. Invest.*, 1941, **20**, 99.

[10] Cohn, E. J., *Proc. Am. Philosoph. Soc.*, 1944, **88**, 159.

was approximately the same in the effusion.

Dogs fed increasingly greater amounts of ANTU during frequent intervals develop a tolerance for large doses of the drug. An increase in the serum α globulin concentration appeared to be a characteristic finding in the development of the tolerance. The plasma cholesterol concentration increased markedly during drug administration and diminished when it was discontinued.

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Folic Acid (Pteroylglutamic Acid)* Studies: Hematologic Remissions in Pernicious Anemia.

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Since the *Lactobacillus casei* factor (folic acid) was synthesized in 1945 by Angier and others,[1] many clinical investigators have clearly demonstrated its effectiveness in the treatment of Addisonian pernicious anemia, the anemia of sprue, nutritional macrocytic anemias and the macrocytic anemias of pregnancy. It has likewise been shown that folic acid is quite effective in producing a complete hematologic remission in certain severe macrocytic hyperchromic anemias of infants which are characterized by poor nutrition, a transient histamine fast achlorhydria and a marked megaloblastic hyperplasia of the bone marrow. The treatment of certain human macrocytic anemias with folic acid (pteroylglutamic acid) has been reported by Darby, Jones and Johnson,[2] Spies and co-workers,[3] Moore and co-workers,[4] Goldsmith,[5] Doan, Wilson and Wright,[6] Zuelzer[7] and others. These extensive studies have been made possible by the chemical identification and synthetic preparation of folic acid (pteroylglutamic acid) in sufficient amounts to permit extensive clinical investigation in several fields. Within the past 12 months we have used synthetic folic acid in treating a great number of different blood dyscrasias. The full report of our investigations will be published in a later communication.

This report deals with the effectiveness of folic acid in the treatment of 4 untreated cases of classical Addisonian pernicious anemia in severe relapse. All of the patients in this group were hospitalized for thorough study and control observations prior to the institution of folic acid therapy. In addition to the usual laboratory procedures for classifying and studying an anemia, sternal marrow aspirations, complete X-ray and fluoroscopic studies of the gastrointestinal tract and gastric analyses were done on each patient. None of these patients exhibited involvement of the central nervous system. A typical hematologic remission is graphically illustrated by the erythrocyte, hemoglobin and reticulocyte response indicated in Chart 1.

* The synthetic folic acid (Folvite) used in this clinical investigation was kindly supplied by Stanton M. Hardy, M.D., Medical Director, Lederle Laboratories, American Cyanamid Company, Pearl River, N.Y.

† The authors wish to express their gratitude to Miss Helen May Holt for her technical assistance in this investigation.

[1] Angier, R. B., and others, *Science*, 1945, **102**, 227.

[2] Darby, W. J., Jones, E., and Johnson, H. C., *J. A. M. A.*, 1946, **130**, 780.

[3] Spies, T. D., Vilter, C. F., Koch, M. B., and Caldwell, M. H., *South. M. J.*, 1945, **38**, 707.

[4] Moore, C. V., Bierbaum, O. S., Welch, A. D., and Wright, L. D., *J. Lab. and Clin. Med.*, 1945, **30**, 1056.

[5] Goldsmith, G. A., *J. Lab. and Clin. Med.*, 1946, **31**, 1186.

[6] Doan, C. A., Wilson, H. E., Jr., and Wright, C. S., *Ohio State M. J.*, 1946, **42**, 139.

[7] Zuelzer, W. W., *J. A. M. A.*, 1946, **131**, 7.