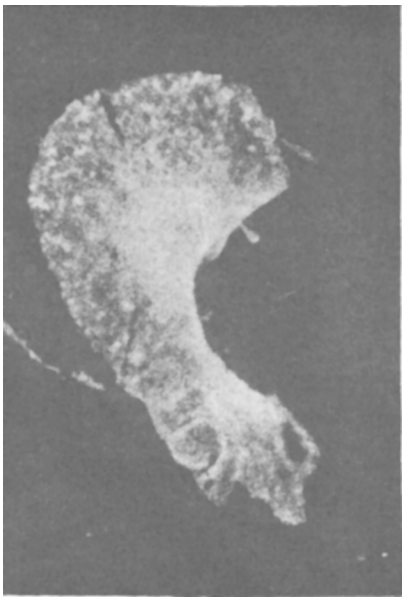

FIG. 5. Autoradiograph of a kidney of a mouse that received probenecid before labelled penicillin.

kidney of a mouse that received 0.2 mg of probenecid per g weight *per os* 2 hours before the injection of penicillin. The glomeruli are visible against a background of relatively penicillin-free tubuli, while in the case of animals that received only penicillin the renal tubuli show a very high degree of activity.

Summary. Radioactive S^{35}-labelled penicillin has been injected into mice and its distribution in the various tissues of the body studied by autoradiographical technics. The results correlate well with the observations that have been made using the microbiological assay for penicillin. In the kidneys the concentration is always, and in the liver generally, higher than in the blood, but in other organs during the whole course of distribution and excretion it is lower than in the blood. In the lungs the concentration nearly approximates that of the blood, while in most tissues it is considerably lower. It is lowest in the central nervous system. The method here described can be applied to the study of penicillin distribution in diseased tissues. Preliminary observations have shown the diffusion of labelled penicillin into abscesses, and the difference in distribution of penicillin in the kidneys under the influence of probenecid and after the administration of penicillin alone.

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Plasma Lipoprotein Changes Following Thermal Injury in the Dog.* (20949)

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Following burn injury, there is consistently a rise in the α-globulin fraction of plasma in human beings, dogs, and various other animals, as shown by electrophoretic studies (1-4). The fact that, in dogs, the α-globulin fraction contains all of the plasma lipoproteins(5,6) suggested to us that these lipoproteins might be considerably altered by thermal injury. Further support for such a relationship comes from chemical fractionation studies(7).

In the present investigation, the lipoproteins were isolated from the plasma of burned dogs and characterized by flotation in an analytical ultracentrifuge. Marked changes in the lipoprotein fractions were shown to

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occur as a consequence of thermal injury.

Methods. Five dogs were maintained for at least 2 weeks prior to the experiment on a standard diet of dog biscuit, and were deprived of food for 12 hours immediately preceding the experiment. The dogs were anesthetized with nembutal and burned by a hot water scald. Three animals were immersed for 30 seconds to the axillae in water at 70°C. The hind legs of a fourth dog were immersed in water at 80°C for 30 seconds. A fifth dog, a control, was not burned but was otherwise treated similarly to the other animals, *i.e.*, anesthetized with nembutal, and then bled at intervals and deprived of food for 72 hours. The control factors, therefore, included anesthesia, the fasting of the experimental dogs due to loss of appetite after injury, and blood loss due to sample collecting. Samples of blood, 20-30 ml in volume, were drawn into oxalated tubes immediately preceding burning and at several intervals up to 72 hours afterward. A biuret determination of total plasma protein and a hematocrit determination were made, and the high and low density lipoproteins were separately isolated from each plasma sample. The low density lipoproteins were isolated and characterized by a technic similar to that of Gofman *et al.*(8). Seven ml of plasma were diluted with 2 ml of an approximately 3.4 M potassium bromide solution, which was standardized to give a resultant protein-free solution density of 1.063, and the preparation was centrifuged at 130,000 x g for 12 hours in a Spinco Type C rotor. The top one ml in the centrifuge tube, containing the low density lipoproteins, which floated, was pipetted off for examination in the analytical ultracentrifuge. The high density lipoproteins were isolated from the bottom fraction of the low density preparation. This bottom fraction, which contained the proteins that sedimented in a medium of density 1.063, was thoroughly mixed and a 4 ml sample removed. To this sample were added 5 ml of an approximately 4.1 M potassium bromide solution, such that the new protein-free solution density was 1.21. This preparation, similar to that of Lewis and coworkers(5), was centrifuged at 130,000 x g for 24 hours and the top one ml, containing the high density lipoprotein, was removed for ultracentrifugal analysis. As can be seen from volume considerations, the low density lipoprotein was concentrated 7-fold and the high density lipoprotein by a factor of 3.5 over the original concentration in the plasma.† The increased resolution of components of the low density lipoprotein during the analytical run, made possible by higher concentration and lower solvent density, was the principal reason for isolating the low density lipoprotein separately from the high density lipoprotein. The analytical runs were made in a Spinco model E ultracentrifuge at 260,000 x g at 25-26°C. The low density lipoproteins, in a solvent of density 1.063, were characterized by their S_f values, in accordance with the technic and nomenclature of Gofman and co-workers(8). The high density molecules, in a solvent of density 1.21, were characterized by —S values following Lewis *et al.*(5). Two-channel cells, similar to those described by Milch (9), were used in most of the runs. These cells allow the base line to be directly superimposed on the lipoprotein schlieren pattern. Lipoprotein concentrations were calculated from the areas of pattern tracings, using the value 0.00154 $(g/100\ cc)^{-1}$ for the specific refractive increment.

Results. Table I indicates the changes from pre-burn values of the plasma concentrations of high and low density lipoprotein and total (biuret) protein. It also shows the relative distribution of lipoprotein in various S_f or —S classes for each pre- and post-burn sample. The S_f or —S classes were arbitrarily chosen to best show the changes in distribution.

It will be seen that 2 major changes common to all 4 experimental animals following thermal injury are a shift of the high density lipoprotein to faster flotation rates and a marked increase in the concentration of the low density lipoprotein. The normal high density lipoprotein consisted, in all dogs studied, principally of a single component with —S 3, and a smaller component, often unresolved, at —S 6. Following thermal in-

† A few plasma samples were less than 7 ml. These were made to volume with isotonic sodium chloride solution.

TABLE I. Concentration and Distribution in Flotation Rate Classes of Plasma Lipoprotein from 4 Dogs with Thermal Injury and a Control Dog.

	Absolute values of pre-burn protein conc. (g/100 cc)				% of pre-burn protein concentrations			% distribution of lipoprotein in each sample‖						
								High D			Low D			
	High D*	Low D	Total plasma	Time after thermal	High D	Low D	Total plasma	L (–S class)			L (S_f class)			
Dog	L	L	protein	injury (hr)	L	L	protein	0-4	4-8	8-14	0-5	5-10	10-25	25-50
A†	.48	.06	—	Before	100	100	—	71	25	4	—	—	—	—
				4.5	100	200	—	56	38	6	—	—	—	—
				7.5	100	170	—	50	44	6	—	—	—	—
				25	130	220	—	34	51	15	—	—	—	—
				30	110	190	—	33	51	16	—	—	—	—
B‡	.72	.08	6.7	Before	100	100	100	88	12	0	75	25	0	0
				6	89	130	87	84	16	0	76	24	0	0
				23	—	140	90	71	22	5	66	34	0	0
				50	110	250	93	60	34	6	80	17	3	0
C‡	.54	.23	6.1	Before	100	100	100	62	36	2	73	25	2	0
				6	120	120	102	68	29	3	71	23	6	0
				25	130	160	100	51	43	6	44	38	17	1
				48	—	190	100	33	54	13	23	34	35	8
D‡	.58	—	6.2	Before	100	100	100	88	12	0	33	56	11	0
				25	120	170	103	57	36	7	20	36	44	0
				48	110	240	98	51	39	10	27	59	14	0
				71	94	—	103	68	22	10	—	—	—	—
E§	.57	—	6.2	Before	100	100	100	85	15	0	51	43	6	0
				25	110	70	103	88	12	0	53	47	0	0
				47	86	90	97	87	13	0	49	44	7	0
				71	100	100	103	88	12	0	68	29	3	0

* D = density; L = lipoprotein.
† Hind legs immersed in water at 80°C for 30 sec.
‡ Immersed to axillae in water at 70°C for 30 sec.
§ Control dog.
‖ Total concentration of high or low density lipoprotein in each sample is taken to be 100%.

jury, the principal normal component slowly decreased, while a polydisperse distribution of components, with flotation rates up to —S 12-13, increased. There appears to be no consistent trend of total concentration changes in the high density lipoprotein of the experimental dogs, although there is considerable fluctuation in both the experimental and control values. Apparently the decrease in the normal component roughly equaled the increase in the new components. This is illustrated in Fig. 1a-1b. These changes appear to be reversible. In one dog, D, which recovered from the thermal injury, the distribution started to shift back toward normal by 71 hours.

The low density lipoprotein in unburned dogs was present in rather low concentration compared to that of the high density lipoprotein, and consisted of a polydisperse group of components of S_f 1-10 with the largest components at S_f 3-5. After thermal injury, however, the concentration of the low density molecules approximately doubled. This increase is not ascribable to a hemoconcentration effect because the total plasma protein values remained essentially constant. Furthermore, in dog B, the total plasma protein concentration actually decreased while the low density lipoprotein concentration increased.

A variety of distribution pattern changes for the low density lipoprotein were observed in the 4 dogs following thermal injury, as may be seen in Fig. 1d-h. In dog B (Fig. 1d-f), a slight increase in faster components, S_f 5-10, occurred at 23 hours, but by 50 hours a large increase in a slow component, S_f 4, had occurred. In dog C (Fig. 1g-h), a large increase of faster components occurred and progressed continuously. The distribution changes seen in dog A were somewhat similar

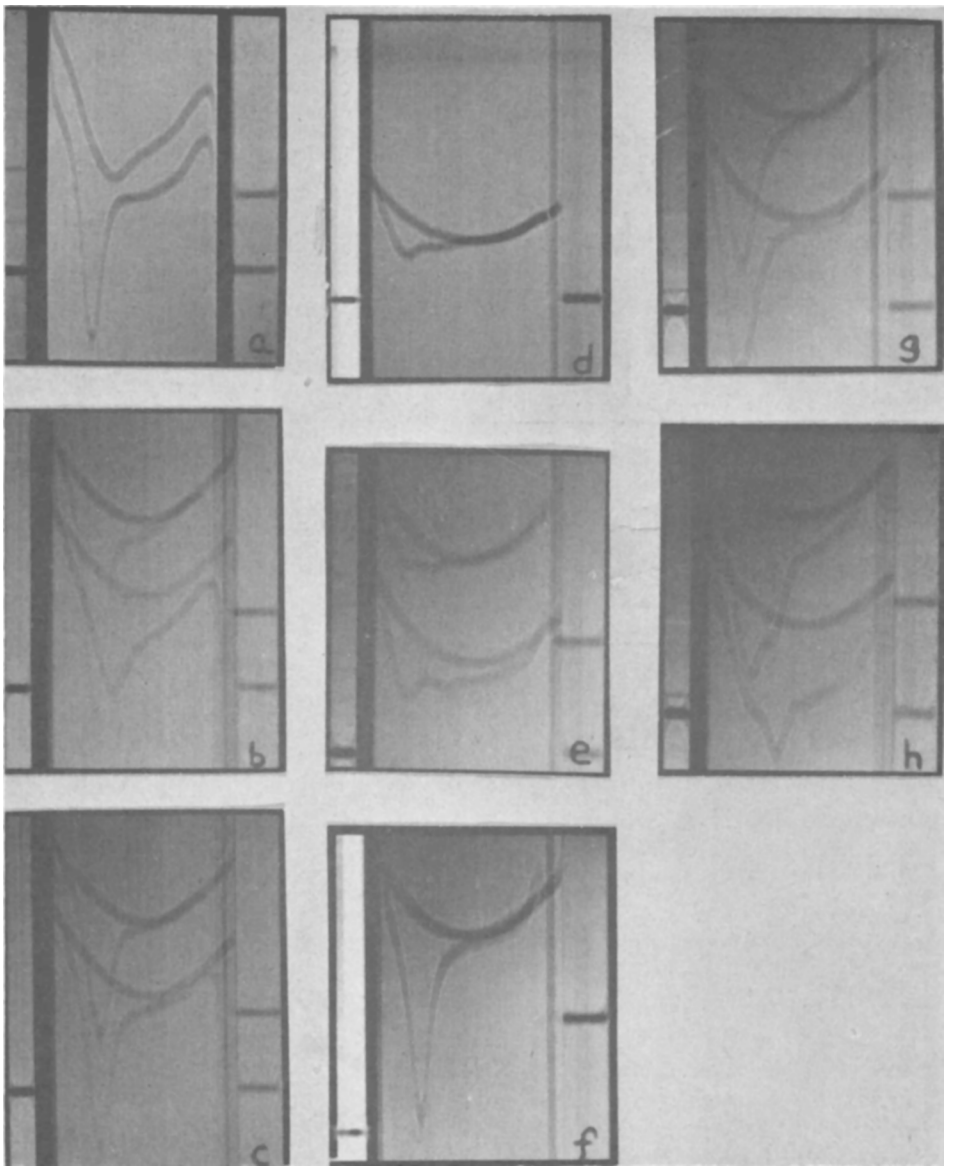

FIG. 1. Ultracentrifuge patterns of plasma lipoprotein from dogs before and after thermal injury.

a. High density lipoprotein from dog A. Upper pattern, 30 hr after burn; lower pattern, before burn. Conc. 4× from plasma.

b. High density lipoprotein from dog D. Upper pattern, before burn; lower pattern, 48 hr after burn. Conc. 3.5× from plasma.

c. High density lipoprotein from dog E (unburned control). Upper pattern, 71 hr after initial sample; conc. 2.5× from plasma. Lower pattern, initial sample; conc. 3.5× from plasma.

d. Low density lipoprotein from dog B, before burn. Conc. 7× from plasma.

e. Low density lipoprotein from dog B. Upper pattern, 6 hr after burn; conc. 4× from plasma. Lower pattern, 23 hr after burn; conc. 7× from plasma.

f. Low density lipoprotein from dog B, 50 hr after burn. Conc. 7× from plasma.

g. Low density lipoprotein from dog C. Upper pattern, before burn; lower pattern, 6 hr after burn. Conc. 7× from plasma.

h. Low density lipoprotein from dog C. Upper pattern, 25 hr after burn; lower pattern, 48 hr after burn. Conc. 7× from plasma.

Boundaries are moving from right to left in above frames. All runs made at 260000 × g at 25-26°C. a-c taken 64 min. after full speed at a bar angle of 60°. d-h taken 32 min. after full speed at a bar angle of 50°, except for frame d where the bar angle was 60°.

to those of dog C.‡ In dog D, the changes were intermediate between those of dogs A and C in that both a pronounced shift to faster components and an increase in a slow component occurred.

Essentially no changes were seen in either the high or low density lipoproteins of the control dog. Although there was some fluctuation in the total concentration of both the high and low density lipoprotein, there was no specific trend. The distribution of both types of lipoprotein remained completely unchanged. The high density lipoprotein pattern of this dog is shown in Fig. 1c.

Discussion. While these results must be considered preliminary owing to the small number of dogs studied and to technical difficulties which produced gaps in the data, they are sufficient to show that substantial changes do occur in the plasma lipoproteins of the dog following thermal injury.

The increase in concentration of the low density lipoproteins is probably metabolically related to the shift of the high density lipoprotein to faster flotation rates. However, this increase in concentration of the low density molecules is probably not due simply to the inclusion of rapidly floating high density lipoprotein in the low density lipoprotein fraction. The 2 classes of molecules appear to remain distinct, as indicated in the experiment with dog A. Here, the high and low density lipoproteins were isolated and analyzed together at density 1.21. The preburn distribution of components consisted of components —S 2-7 and —S 23-27. The latter components correspond to the components S_f 1-10 in a medium of density 1.063. After thermal injury the distribution shifted respectively to —S 2-13 and —S 23-40 but there were no components in the range of —S 13-23.

It is difficult to speculate on the significance of these changes since the metabolic mechanisms of plasma lipoprotein regulation are rather obscure at present. However, low density lipoprotein changes similar to the ones reported here have been observed in rabbits subjected to local cold injury(10,11) and total body irradiation(12). The changes probably represent a response of the animal to tissue injury in general, just as do the α-globulin changes which have approximately the same time course and can be produced by many different kinds of injury(1).

The lipoprotein changes are probably not associated directly with the shock syndrome, and appear to occur later. In this investigation, furthermore, the conditions of thermal injury to the dogs were such that they did not go into severe shock. Although the hematocrit increased significantly a few hours after thermal injury, it was back to normal at 24 hours when the lipoprotein changes began to appear, at which time some of the dogs were quite active.

Summary. Plasma lipoproteins of 4 dogs were studied by flotation in the ultracentrifuge before and after thermal injury. After injury, the high density lipoprotein of all 4 shifted to higher flotation rates without consistent changes in concentration. The low density lipoprotein increased in concentration in all 4 dogs with variable flotation rate changes among the 4. No significant changes were seen in the lipoproteins of a control dog. It is suggested that these lipoprotein changes contribute to the electrophoretic α-globulin changes seen generally after injury.

‡ The distributions of low density lipoprotein in samples from dog A are not listed in the table since this low density lipoprotein was run at a density of 1.21 and thus is not comparable quantitatively with the low density lipoprotein of the other dogs.

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Recovery of a Rickettsia of the Spotted Fever Group from *Microtus pennsylvanicus* from Virginia. (20950)

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Certain of the small mammals native to North America, particularly rodents and rabbits, have been suspected of serving as reservoirs of Rocky Mountain spotted fever because (a) they are the natural hosts of the tick vectors of this disease; (b) many are experimentally susceptible to infection with spotted fever; and (c) antibodies against spotted fever rickettsiae have been demonstrated in sera from some of these small mammals. However, despite the fact that the disease has been long studied in the United States, no confirmed strains of spotted fever have yet been reported isolated from wild-caught mammals in this country.

During 1952 and 1953 isolation studies were undertaken at the Army Medical Service Graduate School in an effort to detect the presence of spotted fever rickettsiae among small wild mammals collected in known endemic areas in the vicinity of the District of Columbia. The present paper reports the isolation of a strain of rickettsiae belonging to the spotted-fever group from the tissues of a meadow mouse, *Microtus pennsylvanicus*, collected during this study from a suburb of Alexandria, Va.

Materials and methods. One hundred and five small mammals were collected from yards and fields believed to be sources of recent cases of spotted fever in the vicinity of Alexandria, Va., and of Gaithersburg and Laurel, Md. These animals were trapped alive by means of small metal box traps and were returned to the laboratory for rickettsial isolation studies.

Ten percent suspensions of the brain, spleen and liver from each of the wild-caught animals were prepared in sterile saline and inoculated intraperitoneally into male guinea pigs. A portion of the inoculum in each case was streaked on blood agar plates to determine the presence of contaminating bacteria. The test guinea pigs were examined daily for fever (rectal temperature of 104°F or higher) and signs of disease. Those guinea pigs which became febrile 3 or more days following inoculation were routinely sacrificed on the second or third day of fever and transfer of suspensions of their liver, spleen, testes and tunica vaginalis were made intraperitoneally to fresh guinea pigs and mice and into the yolk sacs of 7-day-old embryonated eggs. Impression smears were prepared from the uncut surfaces of spleen and liver and from scrapings of the tunica vaginalis of passage guinea pigs. These preparations were stained by Giemsa's and Macchiavello's methods and examined microscopically for the presence of intracytoplasmic rickettsia-like bodies. When an infectious agent suggestive of rickettsiae was isolated in guinea pigs, convalescent sera drawn from these animals 21 to 30 days after the subsidence of fever were tested for complement-fixing antibodies against spotted fever rickettsiae. Complement-fixation tests were performed according to the methods employed in the Department of Virus and Rickettsial Diseases of the Army Medical Service Graduate School. Sera from passage guinea pigs were titrated in the presence of specific

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