portance. Lack of rapid and complete equilibrium between the newly formed and injected acetylamino acids would also yield low radioactivity in the excreted product. Both acetate and alanine (pyruvate) carbons can be used in this acetylation.

Summary. Intravenously injected acetyltryptophan and acetylglycine are excreted in part by rats. Advantage was taken of this behavior to trap any radioactive acetylamino acids that are formed *in vivo*. The results show that natural amino acids can be acetylated *in vivo*.

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Distribution of Bacitracin in the Body.* (17394)

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Bacitracin is an antibiotic with a wide antibacterial spectrum. It was discovered in the Bacteriological Research Laboratory of the Department of Surgery of Columbia University. The active principle is produced by the Tracey I strain of *B. subtilis*.[1] Bacitracin has been used clinically in the treatment of many infections which had failed to respond to other antibiotic and chemotherapeutic agents.[2] Because of its clinical importance, it seemed to the authors worth while to determine the distribution of the drug in the different tissues and fluids of the experimental animal following intravenous administration.

First series of experiments. Eleven normal rabbits weighing from 2 to 4 kilos were selected. Bacitracin in a dosage of 3,000 units per kilo of body weight, dissolved in 3 or 5 cc of physiological saline, was injected slowly into the ear vein of each rabbit. One, 2 or 3 hours after the injection the animals were bled to death under nembutal anesthesia. The whole organ or a portion of the tissue was then removed from the skin, heart, lung, liver, skeletal muscle, brain, bone marrow, pancreas, kidney, intestines and the spleen. Each kind of tissue was ground up in a mortar with an equivalent weight of physiological saline. It was then filtered through cotton. Each filtrate was then assayed for the concentration of bacitracin by the penicylinderplate method against *Micrococcus flavus*.[3] The cerebrospinal fluid, blood, bile and urine were also collected and their bacitracin content similarly determined. The results are presented in Table I.

Discussion. Although there are some wide variations, composite figures as expressed in averages give an approximate value for the various tissues. The figures seem to indicate that the antibiotic is widely distributed throughout the body, but its concentration is somewhat variable in different tissues. For example, the chorioid plexus apparently acts as a barrier to the passage of bacitracin. None or only a trace of bacitracin was found in the cerebrospinal fluid or in the brain tissues after intravenous or parenteral injection. This is also true of penicillin and streptomycin.[4,5]

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The bacitracin used was supplied by the Commercial Solvents Corporation of Terre Haute, Ind.

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TABLE I. Concentration of Bacitracin in Body Tissues and Fluids of 11 Rabbits After a Single Large Intravenous Injection of Bacitracin. (3,000 units/kilo body wt).

Tissue or fluid	Units of bacitracin per g or cc												
	Sacrificed after 1 hr				Sacrificed after 2 hr				Sacrificed after 3 hr				
Rabbit No.	1	2	3	4		5	6	7	8		9	10	11	
Wt in kg	2.5	2.0	2.5	2.5	Avg	2.0	2.5	2.5	2.0	Avg	4.0	3.5	4.0	Avg
Blood	3.65	18.0	2.55	10.0	8.55	1.25	1.6	6.2	10.0	4.76	5.1	2.56	3.8	3.82
Bile	1.24	1.8	1.5	1.4	1.46	2.61	0.64	1.8	2.85	1.97	0.93	1.02	0.8	0.9
Cerebrospinal fluid	0.1	0.1	0	0	Trace	Trace	Trace	0.1	Trace	Trace	0	Trace	Trace	Trace
Urine	177.0	57.5	180.0		138.16	325.0	15.0	4.6	65.0	102.4	11.2	78.0	17.0	35.4
Kidney	3.9	3.1	1.98	5.4	3.59	2.61	1.41	4.2	12.4	5.15	6.0	5.4	2.8	4.7
Lung	1.35	2.0	0.7	1.25	1.32	0.45	0.25	1.35	5.4	1.86	0.8	0.62	1.32	0.91
Pancreas	0.1	0.7	0.34	0.31	0.36	0.28	0.1	0.32	1.84	0.63	1.2	0.4	0.38	0.66
Skeletal muscle	0.53	0.7	0.1	0.23	0.39	0.1	0.1	0.29	1.53	0.5	0.42	0.31	0.1	0.27
Heart	0.36	1.35	0.3	0.75	0.69	0.3	0.1	0.43	1.08	0.47	0.42	0.29	0.34	0.35
Spleen	0.39	0.9	0.1	0.48	0.47	0.1	0.1	0.23	0.6	0.26	0.44	0.31	0.24	0.33
Liver	0.27	0.72	0.1	0.48	0.39	Trace	0.1	0.51	0.96	0.39	0.44	0.1	0.26	0.27
Brain	Trace	0	0	Trace	Sl. Tr.	Trace	0	Trace	0	Sl. Tr.	Trace	0.1	0	Trace
Bone marrow	0.84	0.72	0.66	0.91	0.78	0.7	0.54	0.3	3.12	1.16	0.96	0.41	0.7	0.69
Skin	0.82	3.6	0.38	0.65	1.36	1.05	0.12	0.5	1.7	0.84	0.58	0.13	0.48	0.39
Large intestine	0.42	2.85	Trace	0.80	1.0	0.1	0.1	0.67	1.92	0.69	0.24		0.22	0.23
Small intestine	0.33	0.33	0.34	0.42	0.35	0.1	0.1	0.24	1.0	0.36	0.28		0.42	0.35

However, this barrier is overcome when the meninges are severely inflamed, as in suppurative meningitis, for then a significant level of bacitracin can be recovered in the cerebrospinal fluid.[6]

The high bacitracin content in the kidney is to be expected in view of the fact that this organ is active in the excretion of the drug. The concentration of bacitracin in the urine obviously depends to a considerable degree upon both the total intake and the total output of fluid. The output of urine could not be determined because the animals voided a variable amount during the course of the experiment and the urine was not collected. The concentrations noted in the table were those found in the urine present in the bladder at the time of death. The wide variation in these figures was not unexpected for it is similar to that found in patients following intramuscular injection. Although no albumin or casts were found in these urine specimens and there was no gross evidence of kidney damage at the time of autopsy, it is possible that there may have been some variation in renal blood flow or glomerular or tubular filtration to account for the wide variations in the urine concentrations. This might also explain the wide range in the blood levels.

Of interest and possibly of clinical importance is the relatively high bacitracin content in the lungs, the skin and the bone marrow. The content of the drug in the bone marrow is in contrast to that of penicillin, which according to the report of Struble and Bellows does not reach this tissue.[4] However, some doubt is cast upon this finding with regard to penicillin by the excellent response of acute osteomyelitis to treatment with this drug. This might be explained on the basis of increased permeability of the capillary walls in the early stages of an acute inflammation. The high figure for bile may be partially explained by the fact that a control specimen of bile showed an inhibitory effect against the growth of *Micrococcus flavus*. However the variation in the levels, with the peak average at two hours, suggests that bacitracin is ex-

[6] Teng, P., and Meleney, F. L., accepted for publication in *Surgery*.

TABLE II.
Concentration of Bacitracin in the Chest and Peritoneal Fluids and in the Blood Serum of Rabbits after an Intravenous Injection of the Antibiotic.* (3,000 units/kilo body wt).

Rabbit No.	Bacitracin levels in units/cc		
	Chest fluid	Peritoneal fluid	Blood serum
1	2.3	2.3	1.6
2	5.0		7.5
3	4.2		4.5
4		2.6	2.55
5	2.4		2.8
Avg	3.47	2.45	3.79

* One hour after bacitracin injection and 25 hours after the intraperitoneal and intrathoracic injections of aleuronat-starch.

creted in the bile.

There was a moderate concentration of bacitracin in the wall of both the small and the large intestine. This suggests the possible value of the antibiotic in the treatment of intestinal infections, either bacterial or protozoan, provided that these infections are caused by organisms susceptible to bacitracin.

The liver, spleen, pancreas, heart and skeletal muscles all showed a moderate amount of the drug, which suggests that infections in these organs might be reached by the circulating drug.

Second series of experiments. In the second part of this study, 10 cc of aleuronat-starch, which contained 3% starch and 5% aleuronat, were injected into the pleural and into the peritoneal cavities of 5 normal rabbits 24 hours before the intravenous administration of bacitracin in the same dosages described above. These animals were sacrificed one hour after the injection of the antibiotic. The pleural or peritoneal exudate produced by the irritation of the aleuronat-starch was collected for a study of the bacitracin level by the penicylinder-plate method. The results are shown in Table II.

Discussion. These figures seem to indicate clearly that bacitracin diffuses into the exudate in the purulent response to a chemical irritant in experimental rabbits and presumably will diffuse into an exudate of infectious origin in man. In one rabbit, the bacitracin levels in the peritoneal and pleural fluid were higher than those in the blood serum. In one the level in the chest fluid was lower, and in the others the titres were approximately the same as in the blood serum. Johnson, Anker and Meleney reported that subcutaneous bacitracin could save experimental mice following the intraperitoneal injection of 10,000-100,000 minimum lethal doses of hemolytic streptococci.[1]

In 3 clinical cases of ascites, from 1 to 6 hours after a single intramuscular injection of 49,000 units of bacitracin, which was approximately 1,000 units per kilo of body weight, Michie found that the concentration of the drug varied from 0.0018 to 0.128 unit per cc of ascitic fluid. In the pleural fluid of one patient he demonstrated a level of 0.5 unit per cc 4 hours after an intramuscular injection of 40,000 units of bacitracin.[7]

The fluid collected from the above patients was of a transudative nature. In the case of exudates, a higher bacitracin content might well be expected. Whenever the permeability of blood vessels is increased by an inflammatory process, it is easier for bacitracin to pass through the capillary bed and enter the inflamed tissues.

During the early commercial production of bacitracin, certain lots showed a disturbing degree of kidney irritation which interfered with systemic administration, but since July of 1948 our experience has shown that a product which meets the Food and Drug Administration's specification for toxicity of an LD50 of 500 units for 20-g mice, produces only a minimal and transient degree of kidney irritation. The lot used in these experiments, namely, No. 480408, met that specification and has been used without difficulty by intramuscular injection in a large number of human infections.

Summary. One, 2 or 3 hours after a large single intravenous injection of bacitracin, the concentration of the drug in the different tissues of rabbits was found in decreasing order as follows, subject to some individual variation: urine, kidney, blood, bile, lung, bone marrow, skin, large intestine, pancreas, heart muscle, skeletal muscle, liver, spleen,

[7] Michie, A., work in progress.

small intestine, cerebrospinal fluid and brain.

In the chest and peritoneal exudates produced by aleuronat-starch injection, the titres of bacitracin one hour after an intravenous administration approximated those in the blood.

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Production of an Inactive Derivative of Purified Prothrombin by Means of Purified Thrombin.* (17395)

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The blood clotting mechanism is of such complexity that the reactions which the several clotting factors can participate in may be followed with certainty only when these factors are isolated and obtained in purified form. With the use of such purified preparations, it has been shown that, depending upon the conditions of the experiment, thrombin may have entirely different effects on prothrombin. When small amounts of thrombin are added to prothrombin dissolved in 25% sodium citrate solution, the rate of activation of the prothrombin to thrombin is increased;[1,2] when thrombin and prothrombin react in physiological saline solution the prothrombin is changed to an inactive form in which it does not react to the addition of calcium, Ac-globulin and thromboplastin.[3,4] These effects are apparently produced consecutively when relatively large amounts of thrombin are used. There first appears a decrease in sensitivity to calcium, Ac-globulin and thromboplastin, then a reappearance of the original sensitivity. These experiments might be interpreted to mean that prothrombin is inactivated only to be changed back to its original active form again. It appears more reasonable, however, that thrombin acts first to form a slightly modified prothrombin which is not reactive to thromboplastin, and that this modified prothrombin is then changed to another active form of prothrombin which yields thrombin. The sequence may be described by the following: Prothrombin (sensitive to calcium + Ac-globulin + thromboplastin) → Prothrombin-derivative I (not sensitive to calcium + Ac-globulin + thromboplastin) → Prothrombin-derivative II (sensitive to calcium + Ac-globulin + thromboplastin) → Thrombin and possibly other reaction products. In this paper we shall show that the first prothrombin-derivative, a protein insensitive to calcium + Ac-globulin + thromboplastin, can be identified by electrophoresis.

Experimental procedures. A large homogeneous supply of prothrombin was obtained by pooling several preparations made as previously described.[4-6] Many experiments were performed but all of those described in this paper were performed on this pooled sample of prothrombin.

When dissolved in saline solution it retained at least 95% of its activity for 6 hours at room temperature. On electrophoresis the patterns (Fig. 1) showed that 95% of the total protein was present in one component in the descending boundary, while 87% was present in the ascending boundary. The impurities present in the prothrombin sample were thus shown to be sufficiently reduced to

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