ported here show that penicillin in amounts varying between 0.0039 and 0.0625 Oxford units sterilized an inoculum containing 1,000 to 10,000 cells of all the strains tested belonging to serological groups A through N with the exception of group D strains which required 2.0 to 5.0 units to inhibit completely the growth of a similar sized inoculum. Group M strains were the most sensitive; group A, C, G, H, and L strains were next; and group B, E, F, K, and N strains were the least sensitive of those found susceptible to the action of penicillin in small amounts.

From our experience as well as that of others,[11,12] it appears that with the doses and methods of administration currently used in patients, concentrations of more than 1 Oxford unit per cc of blood would be difficult to maintain for an appreciable length of time. It, therefore, seems unlikely that human infections with group D streptococci that require systemic treatment would respond readily to penicillin therapy in amounts commonly used now. Possibly localized infections, where higher concentrations of penicillin could be maintained, might respond favorably to this drug. On the other hand, infections with strains of groups A, B, C, E, F, G, H, L, K, M, and N would be expected to respond favorably to penicillin since they are susceptible in concentrations that can be readily maintained in either the circulation or locally.

By the methods used, no penicillinase or inhibitor of penicillin could be demonstrated in either the extracted cells or filtrate of a broth culture of a resistant group D strain, although with similar methods an inhibitor which was destroyed or inactivated by heat (65°C for 30 min.) was demonstrated in both the cells and filtrate of a broth culture of a strain of *E. coli*. No attempts were made to further identify this inhibitor. If group D streptococci do not produce substances which destroy or inactivate penicillin, they probably will not interfere with the action of the drug on other penicillin-susceptible microörganisms when found in mixed infections.

11 Watson, R. F., Rothbard, S., and Swift, H. F., *J. Am. Med. Assn.*, 1944, **126**, 274.

12 Rammelkamp, C. H. and Keefer, C. S., *J. Clin. Invest.*, 1943, **22**, 425.

14701

Effect of Ether Extract of the Thymus and Pancreas on Synthesis of Acetylcholine.*

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It was found that less acetylcholine was synthesized in the presence of serum[1,2] and spinal fluid[3] from patients with myasthenia gravis than from healthy persons or patients with diseases other than myasthenia gravis. The pathological changes demonstrable at autopsy may be an enlarged thymus and lymphocytic infiltration of certain tissues. Patients with myasthenia gravis may benefit from removal or irradiation of the thymus. It was ascertained in the following that the synthesis of acetylcholine is modified by the presence of thymus extracts.

Experimental. Series I. The effect of minced thymus on the synthesis of acetylcholine *in vitro* was studied in a few experiments, but the results were inconstant. Since the various constituents of the minced thymus

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1 Torda, C., and Wolff, H. G., *Science*, 1943, **98**, 224.

2 Torda, C., and Wolff, H. G., *J. Clin. Invest.*, 1944, **23**, 649.

3 Torda, C., and Wolff, H. G., *Science*, 1944, **100**, 200.

TABLE I.
Effect of Ether Extracts of Various Tissues on the Synthesis of Acetylcholine *in vitro*.

Substance	No. of separate extracts	No. of exp.	Amount of acetylcholine synthesized in % control*			
			Free acetylcholine In presence of frog brain and human		Total acetylcholine In presence of frog brain and human	
			spinal fluid	serum	spinal fluid	serum
			(1)	(2)	(3)	(4)
Controls†		50	100	100	100	100
Thymus	10	20	65	63	62	64
Pancreas	5	10	58	59	54	56
Lymph gland	5	10	100	97	100	106
Thyroid	5	10	113	105	112	108
Salivary gland	4	8	97	99	105	109
Lung	3	6	102	98	104	105
Subcutaneous fat	2	6	103	105	105	110

* The S.E. of the mean for each value was less than ±5%.

† The amount of acetylcholine synthesized in μg per 100 mg of frog brain in the control mixtures and the S.E. of the mean was: (1) 2.11 ±0.053; (2) 1.45 ± 0.011; (3) 3.16 ± 0.049; (4) 2.08 ± 0.029.

may have different or opposing effects on the synthesis of acetylcholine, an ether extract of the thymus was prepared by the method described by Novinski.[4] Fresh minced thymus was immersed in acetone for 6 hours, then the tissue was extracted in a continuous ether extractor for 24 hours. The tissue was discarded and the ether extract evaporated to dryness. Similar extracts were prepared from pancreas, thyroid, lymph gland, salivary gland, lung, and subcutaneous fat.

The synthesis of acetylcholine was studied by the method of Quastel, Tennenbaum, and Wheatley[5] with minor modifications.[2] Two to 5 mg dry extract were suspended in a mixture containing 100 mg minced fresh frog brain, 3 mg physostigmine salicylate, 4.8 mg glucose, 2 cc Ringer's solution, and 1 cc human serum or spinal fluid. The pH of the mixtures was adjusted to 7.4. Identical mixtures without the dry extracts served as controls. The mixtures were shaken and incubated aerobically for 4 hours. After incubation the amount of free and total acetylcholine synthesized was assayed biologically by the use of the sensitized rectus abdominis muscle of frog.

The amounts of acetylcholine synthesized in the presence of the extracts are summarized in Table I. While extracts of thymus and pancreas depressed the synthesis of acetylcholine, extracts of the other tissues did not modify it. It is not likely that the decrease found in the presence of the extracts of thymus and pancreas was due to impurities introduced during the extraction, since extracts of the other tissues did not modify the amount of acetylcholine synthesized.

The nature of the factor responsible for the decrease in the synthesis of acetylcholine is not yet known. Dr. W. H. Summerson was kind enough to ascertain the ultraviolet absorption spectrum of some extracts. The extracts did not have a maximum of absorption between 2,400 and 3,000 Å, therefore they did not contain a demonstrable amount of either nucleoproteins or most of the decomposition products of nucleoproteins.

Series II. In the following series of experiments cats and rabbits were anesthetized with a Dial-Urethane solution (Ciba).† Blood samples were collected from the femoral artery. An equal volume of Locke's solution was introduced into the femoral vein. 150 mg of the dry extract of the thymus, pancreas or lung dissolved in 1 cc of ether, or 1 cc of ether alone, were injected subcutaneously.

[4] Novinski, W. W., *Biochem. Z.*, 1930, **226**, 415.

[5] Quastel, J. H., Tennenbaum, M., and Wheatley, A. H. M., *Biochem. J.*, 1936, **30**, 1668.

† The Dial-Urethane solution when added to the mixtures before incubation did not modify the synthesis of acetylcholine *in vitro*.

TABLE II.
Effect of Injection of Ether Extract of Various Tissues on Properties of Serum.

Animal	Extract injected in ether	No. of exp.	Amt of acetylcholine synthesized in the presence of serum taken after injection of the extract expressed in percent of the amount of acetylcholine synthesized in the presence of serum taken before injection (= 100% base line)* Time of collection of blood (hr) following injection ½	1	2	3	4-6	7-10	22
			Free acetylcholine						
Cat	thymus	4	95	72	66	64	60	66	102
Rabbit	"	2			62		57		96
Cat	pancreas	3		74	64	66	62	71	101
"	lung	3		96	105	95	102	107	101
"	(ether)	4		99	97	100	103	104	102
Rabbit	"	2			62		55		94
			Total acetylcholine						
Cat	thymus	4	98	79	67	61	63	66	99
Rabbit	"	2			62		55		94
Cat	pancreas	3		75	60	62	59	73	97
"	lung	3		98	103	94	98	105	108
"	(ether)	4		100	96	100	102	103	100
Rabbit	"	2		101		99	100		

* The S.E. of the mean for each value was less than $\pm$ >5%.

Blood samples were collected hourly for 10 hours and again after 22 hours. The effect of the serum on the synthesis of acetylcholine was studied as above. In these experiments the incubated mixtures contained 100 mg frog brain, 3 mg physostigmine salicylate, 4.8 mg glucose, 1 cc cat serum, and 2 cc Ringer's solution.

The results are summarized in Table II. The average of the amounts of acetylcholine synthesized in the mixtures containing serum collected before injection of the extracts served as the 100% base-line. The synthesis of acetylcholine did not change significantly in the presence of serum collected during the period of 22 hours from cats injected with ether and with the ether extracts of lung. However, in the presence of serum collected over a similar period following the injection of extracts of thymus and pancreas, the synthesis of acetylcholine was decreased as much as 45%. Serum collected 22 hours after the injection no longer reduced the synthesis of acetylcholine.

Discussion. The above results indicate that ether extracts of thymus and pancreas contained some substance in the presence of which less acetylcholine was synthesized. Serum of cats injected with ether extracts of thymus and pancreas exerts a similar effect on the synthesis of acetylcholine. The serum of the injected cats was probably not a simple mixture of the extract and serum. It is probable that the composition of the serum actually was changed since samples collected from 6 to 10 hours after injection exerted a greater effect than samples collected earlier. It is possible that the factor responsible for the decrease of acetylcholine synthesis is more readily soluble in ether than in water. Our experiments, however, do not exclude the possibility that the substance is ubiquitous and the extraction by ether may have served only to separate it from many impurities, which, by themselves, had some effect upon the synthesis of acetylcholine.

Traces of the extracts of thymus and pancreas were sufficient to reduce the synthesis of acetylcholine to a degree comparable to the decrease of synthesis found in the presence of serum[1,2] and spinal fluid[3] from patients with myasthenia gravis. Therefore it is possible that a decrease of the synthesis of acetylcholine may occur if this factor be released from the thymus into the blood stream.

Summary. 1. The effects of ether extracts of thymus, pancreas, lymph gland, thyroid, salivary gland, lung, and subcutaneous fat on the synthesis of acetylcholine were investigated. 2. Extracts of thymus and of pancreas

significantly decreased the synthesis of acetylcholine *in vitro* while the other extracts had no demonstrable effect on this process. 3. Less acetylcholine was synthesized in the presence of serum from animals injected with the extract of thymus and pancreas than in the presence of serum from non-injected animals or animals injected with extracts of lungs. 4. The extracts of the thymus and pancreas contained neither nucleoproteins nor their decomposition products in an amount demonstrable with ultraviolet spectrography.

14702

Studies on Heavy Metal Poisoning: I. The Use of Natural Radioactivity for Tracer Studies on Uranium.*

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Introduction. Since uranium was introduced as an experimental nephropathic agent by Leconte[1] several attempts have been made to develop a method to measure small quantities of uranium such as are encountered in biological materials in animals poisoned with this heavy metal, but none of these methods has utilized the natural radioactivity of uranium as the basis. Recent developments in the measurement of emanations from radioactive substances have made this possible and with the aid of Dr. A. E. Ruark in the Physics Department a reasonably satisfactory method has been developed.

Our immediate interest in the problem arose from studies by Donnelly and Holman[2] in which it was shown that the intravenous administration of sodium citrate protected dogs against a lethal dose of uranium nitrate. If a method could be perfected for determining small quantities of uranium, it might be possible to determine whether sodium citrate leads to increased elimination of the heavy metal by altering the solubilities of the complex uranium salts, or otherwise. Before these studies could be completed they were interrupted by one of us (W.A.D.) being called away to participate in the war effort. The results to date have indicated: (1) that the method has merit, (2) that uranium nitrate disappears rapidly from the blood stream after intravenous injection, and (3) that approximately ⅔ of the uranium can be recovered in the urine in the first 24 hours after injection.

Methods. Four adult dogs fed the regular kennel diet and allowed free access to water were used. Two of these dogs received daily intravenous injections of sodium citrate (0.33 cc of a saturated aqueous solution [approximately 230 mg] per kg) for 5 days before the injection of uranyl nitrate and for 5 additional days following the injection of the heavy metal. Each of the 4 dogs received an intravenous injection of 5.0 mg/kg of uranyl nitrate ($UO_2(NO_3)_2 \cdot 6H_2O$, Merck in 0.5% aqueous solution) accurately transferred by washing out the syringe with blood. Immediately after the injection of uranium nitrate the dogs were catheterized and placed in clean metabolism cages. All urine passed during the next 24 hours was collected. In each case the 24-hour urine output was diluted up to 1500 cc with cage washings. Blood samples were taken immediately before and at 4, 19, 34, 64, 124, and 244 minutes after injecting the heavy metal—also at the end of 24 hours. The blood samples consisted of 12 cc of venous blood (jugular vein) mixed with 2.0 cc of 1.4% sodium oxalate in a 15 cc graduated centrifuge tube and centrifuged at 3,000 r.p.m. for 35 minutes.

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1 Leconte, *Gas. Med. Paris,* 1854, IX, 488, (cited by MacNider, W. deB.), *Physiol. Rev.,* 1924, **4**, 595.

2 Donnelly, G. L., and Holman, R. L., *J. Pharm. and Exp. Therap.,* 1942, **75**, 11.