METHODS

The penicillin fermentations were conducted in the manner described by Koffler, Emerson, Perlman and Burris[2]; the penicillin essays were made according to the method of Schmidt and Moyer.[3] The following media were used:

Medium I (synthetic medium)

	g
Lactose	20.0
Dextrin	5.0
Glacial acetic acid	4.0
NH_4NO_3	6.0
KH_2PO_4	1.5
$MgSO_4 \cdot 7H_2O$	0.25
$ZnSO_4$	0.04

Distilled water to one liter
pH adjusted to 6.0 with KOH

Medium II (corn steep liquor medium)

	g
Corn steep liquor solids	30.0
Crude lactose	30.0
$NaNO_3$	3.0
KH_2PO_4	0.50
$MgSO_4 \cdot 7H_2O$	0.25

Distilled water to one liter
pH 4.2 to 4.5, unadjusted

The inoculum consisted of 1 ml of a suspension of mold spores. The corn steep liquor solids were ashed in an electric furnace at 1400° F for 4 to 5 hours. No attempt was made to dissolve the ash before it was added to the medium.

RESULTS

Table 1 shows that supplements of corn steep ash significantly increased penicillin production by *P. chrysogenum* strains NRRL1951–B25 and X1612 in the synthetic medium. Both strains of the mold produced more penicillin in the synthetic medium supplemented with 500 mg of ash than in the usual medium containing corn steep liquor. The pH of the fermentations was always in the range for maximum penicillin production in shaken flasks. There was no apparent difference between the growth of the mold in the synthetic medium and that in the synthetic medium supplemented with ash. Growth appeared at approximately the same time in both media.

TABLE 1

THE EFFECT OF CORN STEEP LIQUOR ASH ON PENICILLIN PRODUCTION IN SYNTHETIC MEDIUM

Mold	Medium	Ash in medium	Reaction of medium days				Penicillin days			
			5	6	7	8	5	6	7	8
		mg/100 ml	pH				Oxford units/ml			
NRRL–1951–B25	I	...	7.80	7.87	7.74	7.55	12	19	31	39
		10	7.81	7.96	7.80	7.64	15	27	37	42
		100	7.58	7.67	7.60	7.74	34	45	50	53
		300	7.73	7.71	7.68	7.75	42	65	66	60
		500	7.71	7.70	7.70	7.84	46	73	78	77
	II	...	7.73	7.70	7.91	7.86	40	54	68	67
X1612	I	...	7.42	7.61	7.51	7.56	41	44	38	31
		10	7.40	7.60	7.46	7.49	32	34	50	52
		100	7.34	7.69	7.53	7.50	50	57	52	50
		200	7.37	7.72	8.00	7.76	78	86	91	88
		500	7.50	7.79	7.92	7.84	112	132	119	126
		700	7.56	7.77	7.78	7.70	100	105	115	100
	II	...	7.28	7.45	7.61	7.63	69	102	96	80

[2] H. Koffler, R. L. Emerson, D. Perlman and R. H. Burris, *Jour. Bact.*, 50: manuscript in press.

[3] W. H. Schmidt and A. J. Moyer, *Jour. Bact.*, 47: 199, 1944.

Subsequent experiments showed that the addition of supplements of corn steep liquor ash to the usual fermentation medium (medium II) resulted in a 30 to 45 per cent. increase in penicillin production by strains NRRL1951–B25 and X1612.

DISCUSSION AND SUMMARY

The corn steep liquor used in these experiments showed 16.1 per cent. ash. Thus the 3.0 per cent. corn steep solids in medium II contributed 483 mg of ash to the medium, or approximately the amount required for maximum penicillin production in the synthetic medium. It should be noted that maximum penicillin production resulted when the synthetic medium was supplemented with a level of ash much greater than usually is considered necessary for normal mold growth.

The results cited above indicate that minerals play an important role in the production of penicillin. It is probable that corn steep liquor not only provides a source of nitrogen and carbon for the mold but also supplies the mineral element or combination of mineral elements necessary for optimum penicillin production. A difference in the content or balance of mineral elements may explain why some corn steep liquors are superior to others in penicillin fermentations.

A more extensive report of this work will be published elsewhere; further investigations on the role of mineral elements in penicillin production are being continued.

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HEATED, AVIRULENT ANTIGENS FOR COMPLEMENT-FIXATION TESTS WITH CERTAIN ENCEPHALITIS VIRUSES[1]

THE increasing application of the complement-

[1] This study was carried out under the Commission on Neurotropic Virus Diseases, Board for the Investigation

fixation test for the diagnosis of several human encephalitides compels attention to the use of avirulent antigens, since certain active ones may possibly induce disease through accident or carelessness. Avirulent antigens can be prepared by means of ultraviolet light irradiation.[2] These antigens have the same antigenicity, specificity and lack of anticomplementary effect as fresh, virulent antigens. Moreover, they can be lyophilized and kept for a long time, up to two years.[3] However, inactivation with ultraviolet light requires special standardized equipment, which may not be available at all times and in all places.

Tests were made on the application of heat for preparation of avirulent antigens. The earlier "koktoantigens"[4] made by boiling brains suspensions for 30 minutes were inadequate since it was found in this laboratory that by this means the antigenicity was destroyed. By heating at 60° C for 30 minutes, however, avirulent antigens were obtained which retained sufficient antigenicity for practical use.

Ten to 15 cc of virulent antigens prepared as described,[3, 5] were placed in test-tubes and heated at 60° C for 30 minutes. During heating a more or less heavy flocculation took place. The flocculate was removed by centrifugation at 2,500 rpm for 10 minutes in a horizontal centrifuge, or at 6,000 rpm for 30 minutes in an angle head centrifuge. The supernate constituted the antigen, which could be lyophilized.

Heated antigens derived from the following viruses have been tested: St. Louis, Japanese B and Russian spring-summer (Far Eastern) encephalitis viruses; louping-ill and West Nile viruses; Western, Eastern and Venezuelan equine encephalomyelitis virus. The results of the tests are shown in Table 1.

The specificity of the heated antigens tested by cross-reaction was found to be unchanged and the same as that of unheated, virulent antigens. Furthermore, they had not acquired thereby any anticomplementary effect. The tabulated results show that although a loss of antigenic titer was correlated with heating, the antigenic titer after heating was sufficiently high for the viruses of Japanese B, Russian spring-summer and West Nile encephalitis for safe use. The heated antigens of louping-ill and Western equine encephalomyelitis viruses were somewhat lower but still usable; St. Louis and Eastern equine encephalomyelitis viruses could be employed sometimes, *i.e.*, if the initial titer could be made sufficiently high. The Venezuelan equine virus antigen after heating was ineffective. By test, lyophilization of heated antigens did not reduce their titer; they could therefore be handled for preservation or transportation in the same way as the irradiated antigens.

TABLE 1

ANTIGENS HEATED AT 60° C FOR ½ HOUR AND CENTRIFUGED

Antigen of virus of	LD_{50} titer before heating	Mouse inoc. with undiluted heated antigen	Complement-fixing titer of antigen	
			Before heat	After heat
St. Louis encephalitis	10^{-4}	0/10*	1:4	1:2
			1:16	1:2
Japanese B (Nakayama strain) encephalitis	$10^{-1.5}$	0/5	1:64	1:32
			1:128	1:16
	$10^{-3.5}$	0/10	1:16	1:8
			1:64	1:8
Russian spring-summer encephalitis	10^{-7}	0/5	1:16	1:8
	10^{-5}	0/10	1:32	1:16
Louping-ill	10^{-2}	0/10	1:16	1:4
West Nile disease	$10^{-4.5}$	0/10	1:64	1:32
Western equine encephalomyelitis	10^{-5}	0/5	1:64	1:8
	$10^{-6.5}$	0/10	1:32	1:4
Eastern equine encephalomyelitis	$10^{-6.5}$	0/10	1:2	0
			1:16	1:4
Venezuelan equine encephalomyelitis	$10^{-7.5}$	0/10	1:2	0
			1:8	0

* Fractions represent number of mice dead of virus infection over number used.

Summary. A simple method is described for producing avirulent mouse brain antigens for complement-fixation tests in human encephalitides, by application of heat at 60° C for 30 minutes. The heated antigens retain for most of the viruses employed a sufficiently high titer to warrant their use, since they are also specific, not anticomplementary, can be lyophilized and moreover, are avirulent, and thus can be handled with safety.

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THE RATE OF WATER LOSS FROM THE RESPIRATORY TRACT OF MAN LIVING IN A SUBTROPICAL CLIMATE[1, 2]

NEAR the turn of the century direct measurements of water loss from the lungs were made by Galeotti and his associates,[3] Weyrich,[4] Loewy and Gerhartz,[5]

and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army.

[2] J. Casals, *Proc. Soc. Exp. Biol. and Med.*, 49: 501, 1942.

[3] J. Casals, SCIENCE, 97: 337, 1943; *Jour. Bact.*, 50: 1, 1945.

[4] S. Nakagawa, *Z. Immun.-Forsch.*, 39: 563, 1924; J. Takaki, A. Bonis and O. Koref, *Z. Immun.-Forsch.*, 47: 431, 1926.

[5] J. Casals and R. Palacios, *Jour. Exp. Med.*, 74: 409, 1941.

[1] From the Department of Medicine, Tulane University, School of Medicine and Charity Hospital, New Orleans.

[2] Aided by a grant by the Rockefeller Foundation and Helis Institute for Medical Research.

[3] G. Galeotti and E. Signorelli, *Biochem. Zeitschr.*, 41: 269, 1912; *Arch. f. d. ges. Physiol.*, 160: 27, 1914–15; *Biochem. Zeitschr.*, 46: 173, 1912.