concentrations ranging from about 0.00015 M to 0.0005 M (2.5–8.0 mg per cent.).[1]

Fig. 1 shows the activity-substrate concentration curves obtained with mouse brain. In the absence of any salts other than 0.025 M sodium bicarbonate

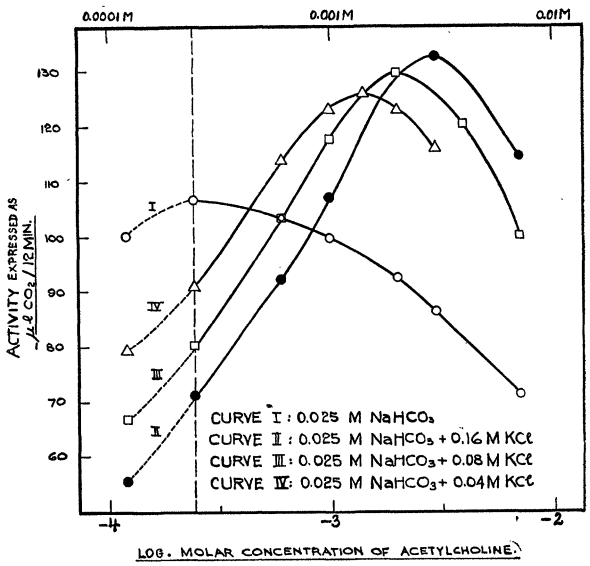

FIG. 1. Hydrolysis of acetylcholine by true cholinesterase as a function of acetylcholine concentration. The broken vertical line marks the 0.00025M (4 mg per cent.) acetylcholine level. The cholinesterase was obtained from mouse brain; 1 g of the tissue was ground and suspended in 5 ml water and 0.2 ml of this suspension was used in all experiments.

(Curve I) the optimum activity is displayed at 0.00025 M (4 mg per cent.) acetylcholine. When potassium chloride in a concentration of 0.16 M is added to the above medium (Curve II) we not only find a shifting of the region of maximum activity from 0.00025 M (4 mg per cent.) to about 0.003 M (49 mg per cent.), but also a pronounced reduction of the enzyme activity at its former optimum of 0.00025 M acetylcholine. Similar effects are obtained with lower concentrations of potassium chloride. In the presence of 0.08 M potassium chloride (Curve III) the optimum substrate concentration is 0.002 M (32 mg per cent.) acetylcholine and the activity at 0.00025 M acetylcholine, though reduced in comparison with the activity found in the presence of 0.025 M sodium bicarbonate alone, is greater than that obtained in the presence of 0.16 M potassium chloride; in the presence of 0.04 M potassium chloride (Curve IV) the enzyme displays its optimum activity at 0.0015 M (24 mg per cent.) acetylcholine, and the decrease in activity at 0.00025 M acetylcholine is still less marked than it is in the presence of the higher salt concentration.

Our experiments show that the relationship between enzyme activity and substrate concentration is changed by the addition of salt to the medium. A stepwise increase in the salt concentration, though not abolishing the excess substrate inhibition as such, causes a gradual shifting of the optimal activity of the enzyme to higher levels of acetylcholine—a fact which brings to light the cause of the discrepancy between Nachmansohn and Rothenberg's results and our own. Furthermore, with increasing concentrations of potassium chloride an absolute increase in the rate of acetylcholine hydrolysis occurs at the new optimum levels and beyond these points as well. Similar results are obtained with true cholinesterase from other sources mentioned previously in this paper.

When sodium chloride instead of potassium chloride is added to the medium in equimolar concentrations, the activity-substrate concentration curves show the same general trend but they are not identical. This fact reveals the possibility of superimposed specific effects of particular ions which will have to be studied later in detail.

The above observations point to the important role ions play in the hydrolysis of acetylcholine by true cholinesterase. The escape of potassium from cells, occurring upon stimulation,[8] might possibly help to maintain optimal conditions for the cholinesterase activity which otherwise, *i.e.*, if the potassium level in the cell remained constant, would drop sharply with declining concentrations of acetylcholine. Conversely, an increase of potassium in the extracellular fluid, shown by various workers[9,10,11] to sensitize cells to submaximal doses of acetylcholine, could cause this effect by retarding the escape of potassium from these cells, thereby creating conditions in which the activity of true cholinesterase towards low concentrations of acetylcholine would be suboptimal.

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THE EFFECT OF CORN STEEP LIQUOR ASH ON PENICILLIN PRODUCTION[1]

IT generally is assumed that corn steep liquor enhances penicillin production because of a specific organic constituent, but during a study of the physiology of *Penicillium chrysogenum* it was found that the inorganic constituents of corn steep liquor played an important role, as will be shown in the following results from preliminary studies.

[8] M. Vogt, *Jour. Physiol.*, 86: 258, 1936.
[9] W. Feldberg and A. Vartiainen, *Jour. Physiol.*, 83: 103, 1935.
[10] G. L. Brown and W. Feldberg, *Jour. Physiol.*, 86: 290, 1936.
[11] D. W. Bronk, *Jour. Neurophysiol.*, 2: 380, 1939.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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