Drug Administration agar diffusion technic for solutions (*6*), using the F.D.A. standard penicillin.

As indicated in Table 1, the order of stability at 5°C. is consistently NaP > NaP cryst. > CaP. CaP and NaP cryst.

TABLE 1

STABILITY OF PENICILLIN SALTS IN UNDILUTED VEHICLES

Vehicle	Penicillin salt	Storage temp. (°C.)	Percentage of activity after storage						
			2 hrs.	1 day	2 days	4 days	7 days	13 days	19 days
Glycerin	CaP	5	1	0					
	NaP cryst.	5	2.5	0.7	0				
	NaP	5	14.5		0.1				
Propylene glycol	CaP	5	1.5	0					
	NaP cryst.	5	4	0.05					
	NaP	5	81	67			21	6	
Carbowax	CaP	5	80	27		16	11	2	
	NaP cryst.	5	102	100		96	90	81	69
	" "	23	102	60		44	30	10	4.7
	" "	37	102	55		18	10	0	
	NaP	5	103	104		98	98	98	92
	"	23	103	100		96	63	38	28
	"	37	103	86		47	23	2.5	

were almost totally and immediately inactivated in glycerin or propylene glycol, while the rate of inactivation was much less in Carbowax; this was qualitatively true for NaP as well, although inactivation was slower than with the other salts.

In check experiments it was found that absolute (but not relative) inactivation rates varied with the commercial source

TABLE 2

STABILITY OF PENICILLIN SALTS IN FRACTIONS OF PROPYLENE GLYCOL DISTILLATION

Portions	Penicillin salt	Percentage of activity after storage at 5°C.							
		2 hrs.	1 day	2 days	10 days	16 days	29 days	38 days	65 days
I	CaP	80	38	15					
	NaP cryst.	93	91		53	14	8	4	
	NaP	98	100		92	90	81	83	68
II	CaP	50	7	3					
	NaP cryst.	98	101		69	35	23	11	
	NaP	95	100		92	92	91	89	82
III	CaP	0							
	NaP cryst.	2	0						
	NaP	31	20	5					

of glycols, and even with different lots from the same manufacturer. Propylene glycol was accordingly distilled, and penicillin stability determined in three portions: I (the first 25 per cent condensed), II (the next 50 per cent), and III (the residue).

All penicillin salts are more stable in I and II and less stable in the residue than in undistilled propylene glycol. In all fractions the stability order is that derived from Table 1.

Our experience is that penicillin stability varies not only with the vehicle and with the particular salt used, but also with the lot of vehicle, whether or not from the same manufacturer. Impurity may be responsible for the latter type of variation. Despite these fluctuations we have found sodium penicillin amorphous most stable and the calcium salt least stable in glycerin and glycols.

Information on penicillin stability is so meager, and in some cases the inactivation so rapid, that assay of the individual preparation is mandatory.

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A Simplified Method of Preparing Active Extracts of B-Glucuronidase[1]

WILLIAM H. FISHMAN and PAUL TALALAY[2]

Departments of Surgery and *Biochemistry, University of Chicago*

The first procedures published (*3*, *4*) for the preparation of active extracts of β-glucuronidase were tedious and often proved to be unsatisfactory. By employing a new method (*1*) of obtaining the enzyme from beef spleen, potent highly purified extracts could be prepared with much less difficulty. However, in two steps of the process (acetone precipitation, evaporation of extracts in an air current) there was danger of loss of activity if a certain amount of care was not employed. Another disadvantage was the need for somewhat lengthy large-scale manipulations. For the past several years, one of us (F.) has routinely employed a simplified procedure for preparing small amounts of the enzyme from rat and mouse organs which does not have the disadvantages of the previous method noted above. Extracts of β-glucuronidase prepared in this way have been used in studying the kinetics of the hydrolysis of phenolphthalein glucuronide (*5*).

Recently, interest has been shown in the use of β-glucuronidase preparations in preference to strong mineral acid for hydrolyzing steroid glucuronides (*6*) in order to avoid destruction of steroid and prevent the production of artifacts (*2*). Accordingly, it was felt desirable at this time to report the method of preparation now in use and to present some data on the degree of purification achieved.

Six rats were killed by a blow on the head, and blood was permitted to drain from the carotids. The livers, kidneys, and spleens were rapidly dissected out. These were pooled separately and were homogenized with 100 cc. of cold water in a Waring blender or in a glass homogenizer, and the homogenate strained through cheesecloth (Step 1). The mixture was acidified to pH 5.0 with 1.0 N acetic acid and kept at 38° for 30 minutes. The proteins which flocculated out were removed by rapid centrifugation (20 minutes at 3,500 r.p.m.), and the supernatant separated and preserved. To this fraction was added an equal volume of saturated ammonium sulfate, the mixture then being centrifuged for 30 minutes. The super-

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[2] Present address: Yale University School of Medicine, New Haven, Connecticut.

natant was removed by suction and discarded. The residuent was dissolved in 20 or more cc. of water (Step 2), an equal volume of saturated ammonium sulfate solution added, and the resultant precipitate centrifuged off and taken up in a small volume of water (Step 3). Glucuronidase activity was determined on specimens removed at Steps 1, 2, and 3 by the method of Talalay, Fishman, and Huggins (*5*). Simultaneous determinations of the protein N of the extracts was also performed (*1*). The results have been arranged in Table 1.

TABLE 1

PARTIAL PURIFICATION OF B-GLUCURONIDASE OF RAT LIVER, KIDNEY, AND SPLEEN

Total weight of organs (grams)	Fraction	Volume (cc.)	Units*/cc.	Glucuronidase activity (total units)	Units/mg. N
34	Liver 1	117	5,700	673,000	1,160
	2	50	6,240	313,000	3,110
	3	20	15,650	313,000	4,280
6	Kidney 1	96	370	35,500	289
	2	25	488	12,300	665
	3	5	1,630	8,150	2,950
4	Spleen 1	100	1,270	127,000	1,270
	2	25	1,990	49,700	22,300
	3	5	7,280	36,300	18,200

* One unit glucuronidase activity will liberate 1 μg. of phenolphthalein in 1 hour at 38°C. from phenolphthalein mono-β-glucuronide at pH 4.5 in 0.1 M acetate buffer (*5*).

Depending on the nature of the tissue being extracted, the activity of the extracts has been increased three to six times, and a 4- to 15-fold purification has been achieved with a preservation of between 23 and 47 per cent of the total original activity. The best purifications were obtained when using spleen, in which the enzyme is present in higher concentration than in either liver or kidney. More powerful preparations were, however, obtained from liver, since this organ contains a greater total amount of enzyme. The application of repeated ammonium sulfate fractionations (*1*) would lead to much purer preparations of the enzyme. Furthermore, the process could be applied unchanged to larger quantities of tissue so that one could achieve even more potent activities. This has been done using a mixture of livers, spleen, and kidneys from 12 animals. However, where it is not important to have highly purified preparations of the enzyme, the product at Step 3 is quite satisfactory.

The present method eliminates the use of an acetone precipitation and the evaporation of the extract in a current of air, two undesirable operations in the original process. The possibility of undue loss of activity here has accordingly been minimized. This method is also more convenient and more rapid than the previous one, since it requires only a few hours for the complete procedure. Good results have been obtained regularly by those inexperienced in enzyme preparative work.

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Frequency Analysis of Electroencephalograms[1]

FREDERIC A. GIBBS and A. M. GRASS

Department of Psychiatry, University of Illinois, and *Neurological Unit, Boston City Hospital*

Many manual and mechanical types of frequency analyzer are possible, and several have been applied to electroencephalographic problems. The majority are objectionable because they use an arbitrary method of integration, but up to the present time none has received general acceptance and most have been discarded by their authors. It can be presumed that they turned out to be either excessively cumbersome or failed to yield significant information.

Hoagland (*9*) used a map measurer to determine the length of the contour of slow components in the electroencephalographic tracing. This system required accurate control of amplitude and did not distinguish between amplitude and frequency.

Coordinate graphic analysis has been applied to the EEG (*5*), but it is such a tedious process that only an inadequate sample is obtainable. For the few seconds of record considered, the method yields an accurate analysis in which phase relations between harmonics are preserved.

Several different types of electrical filters have been used. One system (*10*) contained constant band-width filters in combination with integrators which measured and recorded amplitude simultaneously. This system was an early prototype of Grey Walter's analyzer and yielded essentially the same type of information. A similar system, employed by Davis (*3*) and his collaborators, had filters with a band-width proportional to the frequency. These were inserted into the oscillographic recording channels and the amplitude of the various frequency components observed. Six frequency bands could be recorded simultaneously by both systems.

Many recording frequency analyzers have been built in the past for use in sound-wave analysis (e.g. *2*, *13*).

Before the analyzer designed by one of us (A.M.G., *8*) was built, all previous systems of analysis were considered. Experience with the application of filter systems to electroencephalography convinced the authors that complete integration by electromechanical means was necessary and could be accomplished only by a system which made a repetitive function (continuous belt) of the sample chosen. Simultaneous registration of the oscillographic and analyzed records was sacrificed to obtain the highest possible accuracy. It was intended to incorporate this feature at a later date if important information was gained and if further study showed that a loss of accuracy could be tolerated.

In the past six years the EEG's of over 1,000 normal persons and 700 patients with nervous and mental diseases have been analyzed. The instrument used for this work (*8*) reduces to a spectrum any desired strip of EEG. Such a spectrum is a plot of the alternating-current voltage at any

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