and the needle in the zero position (crossing of the interaural and midsagittal planes) before and after filling of the ventricles with air. From these photographs the coordinates of the lesion are calculated and the place of trepanation is determined.

(3) After trepanation a wire or cannula is introduced through the intact dura, a lesion is produced by thermocoagulation, fluids are aspirated or injected, etc.

This apparatus is being used for psychosurgery. In a series of patients studied in collaboration with H. Freed, lesions have been placed in the region of the medial nucleus of the thalamus (medial thalamotomy) in order to reduce the emotional reactivity by a procedure much less drastic than frontal lobotomy (*2*). The results so far obtained are promising. Further applications of the stereotaxic technic are under study, e.g. interruption of the spinothalamic tract in certain types of pain or phantom limb; production of pallidal lesions in involuntary movements; electrocoagulation of the Gasserian ganglion in trigeminal neuralgia; and withdrawal of fluid from pathological cavities, cystic tumors.

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A Solution for Plasticizing Kymograph Records

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In the past, many solutions such as shellac in ethanol, resin in ethanol or isopropanol, etc. have been used to fix kymograph records. Satisfactory results were not always obtained because, as the solutions became more concentrated due to evaporation, the records were extremely sticky, did not dry rapidly, and had a glossy finish which would not photograph readily.

The solution to be described has been used for 8 months and has always given excellent results in that the records dried within 10 minutes, were never sticky, and always had a dull finish. One further advantage was the ease with which the fixing pan was cleaned. A 10 per cent aqueous solution of sodium carbonate readily removes all traces of the dried fixative from the pan.

The plasticizing solution is composed of cellulose acetate phthalate (Eastman Kodak Co.), 10 grams; ethyl lactate, 150 cc.; ethyl acetate, 150 cc.; and ethanol or isopropanol, 700 cc. To prevent precipitation of the plastic, the solution must be mixed in the following manner: dissolve the cellulose acetate phthalate in 25 cc. of ethyl lactate and then add an equal quantity of ethyl acetate. This solution is added to half of the ethanol or isopropanol with rapid stirring. Some precipitation of the plastic may occur at this point, but the addition of another 25 cc. each of ethyl lactate and acetate will redissolve the precipitate. Add the remainder of the alcohol, ethyl lactate, and ethyl acetate in that order. Filter the solution by gravity through glass wool and bottle. Kymograph records are fixed in the usual manner with this finished solution.

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Yeast Autolysate: A Culture Medium for *Hemophilus influenzae*

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It is generally agreed that *H. influenzae* requires for its growth two distinct factors, the heat-labile V factor and the heat-stabile X factor. Lwoff and Lwoff (*5*) have shown that the V factor is a coenzyme, and their observation has been confirmed by other investigators. Gingrich and Schlenk (*3*) reported that codehydrogenase I replaces the V factor and, in this respect, is superior to codehydrogenase II. The X factor can be replaced by crystalline beef-liver catalase according to Bass, Berkman, Saunders, and Koser (*1*). From the investigations of Hoagland and associates (*4*) as well as of Bass and co-workers (*1*) it is evident that in addition to these two growth factors other compounds may be required for maximal growth. Yeast is known to be rich in V factor; however, it is generally regarded to be lacking in X factor. The experiments reported below revealed that yeast autolysate supplies both growth factors required by *H. influenzae* and suggest that this substrate may be used to advantage as a culture medium and may be useful in studies on the streptomycin sensitivity of this microorganism.

The yeast autolysate (Supplement B) used in these studies was procured from Difco Laboratories. It is a filtrate of autolyzed fresh yeast and, according to Schoenlein (*6*), is rich in coenzyme, glutamine, cocarboxylase, and other B-vitamin factors. Difco Supplement is recommended for use in the cultivation of *Neisseria gonorrhoeae* and has been employed by Buck (*2*). In the present study, two lots (№ 388386 and № 388624) proved to be equally satisfactory. For as yet unexplained reasons another lot (№ 386207) failed to support the growth of *H. influenzae*. The vast majority of experiments were carried out with lot № 388386.

Nineteen strains of *H. influenzae* were used. Six strains were freshly isolated from the spinal fluid of patients with meningitis; the remainder were stock culture strains available in this laboratory or kindly supplied by H. E. Alexander and the Division of Laboratories and Research, New York State Department of Health. Seventeen strains belong to type b and one strain each to types a and c, respectively.

The first series of experiments was undertaken to determine the growth-promoting properties of yeast autolysate. The 19 strains of *H. influenzae* were seeded into 10 per cent yeast autolysate-brain heart infusion. All grew profusely within 24 hours in this culture medium and failed to grow entirely in brain heart infusion used as control. In view of the fact that the inoculum, which was harvested from hemoglobin-proteose peptone № 3 agar, may have contained an adequate amount of X factor, serial transfers were made daily from yeast autolysate-brain heart infusion to yeast autolysate-brain heart

[1] The author wishes to acknowledge the technical assistance of C. F. Crotty.

infusion. Each transfer resulted in a dilution of 1:10. It was found that all strains could be maintained in this culture medium for 15 consecutive subcultures. It is noteworthy that the organisms maintained their type-specificity: specific soluble substance could be demonstrated by means of type-specific antiserum. Moreover, no changes were noted in the morphological and cultural characteristics of the organisms throughout this experiment in films and colony formation on hemoglobin-proteose #3 agar. It may be concluded, therefore, that yeast autolysate in brain heart infusion supplies the essential growth factors required by *H. influenzae*.

The growth-promoting properties of yeast autolysate in different concentrations were then studied. A strain of *H. influenzae* type b, which had been maintained in yeast autolysate-brain heart infusion for 12 consecutive subcultures, was used as inoculum of brain heart infusion containing yeast autolysate in concentrations ranging from 0.01 to 10 per cent. The seeded culture media were incubated at 37°C. The results of this experiment are summarized in Table 1.

TABLE 1

GROWTH OF *H. influenzae* TYPE b AND PRODUCTION OF SSS IN BRAIN HEART INFUSION CONTAINING YEAST AUTOLYSATE

Hours of incubation	Concentration of yeast autolysate (%) 10	1	0.1	0.01	0
	Growth				
18	++++	++++	++	+	−
48	++++	+++	+	−	−
72	++++	+++	+	−	−
	Presence of SSS				
72	++++	++++	+++	+	−

− = no growth, no SSS; + to ++++ = various degrees of visible growth and various amounts of SSS.

It may be seen that *H. influenzae* grew profusely within 24 hours in infusion containing 1 and 10 per cent yeast autolysate while lower concentrations promoted only slight growth. Attention may be called to the fact that the strain produced type-specific substance. Similar results were obtained with several other strains of *H. influenzae*. Yeast autolysate in a concentration of 50 per cent either failed to promote growth or did so more slowly and to a lesser degree than that used in a concentration of 10 per cent.

In order to assay quantitatively the growth of *H. influenzae* in yeast autolysate-brain heart infusion, the following studies were undertaken. A strain of *H. influenzae* type b was grown in 10 per cent yeast autolysate-brain heart infusion for 24 hours. Serial dilutions (from 10^{-1} to 10^{-11}) were then prepared and used for seeding of the same culture medium. In this fashion the smallest inoculum promoting visible growth was determined. It was found that growth was obtained with inocula in dilutions up to 10^{-9}. Similar titrations carried out with other strains revealed that cultures of *H. influenzae* could be diluted from 10^{-8} to 10^{-11} and still promote growth, when used for inoculation of the 10 per cent yeast autolysate-brain heart infusion. Freshly prepared pipettes were used for each dilution. Even so, this method of titration by necessity has only limited accuracy. From the data just presented it appears to be safe to conclude that type b showed at least a 10,000,000- to 100,000,000-fold increase in this culture medium.

Preliminary studies on the comparative efficacy of yeast autolysate-brain heart infusion and hemoglobin-proteose #3 agar (Difco) revealed that growth may be obtained more rapidly in the fluid than on the solid medium, particularly if small inocula are used for seeding purposes. It appears, therefore, that the yeast autolysate medium may be superior under certain conditions to the presently employed hemoglobin-proteose #3 agar. Moreover, incubation under increased CO_2 pressure is unnecessary.

In order to elucidate the question of whether the X factor is supplied by the yeast autolysate or by the brain heart infusion, 10 strains of *H. influenzae* were seeded into 10 per cent yeast autolysate-saline solution. All strains grew within 24 hours and could be maintained in this medium for 18 consecutive transfers. Since each transfer resulted in a dilution of 1:10, it seems safe to assume that X factor, which may have been present in the first inoculum, was no longer left at the end of the experiment. Therefore, yeast autolysate fulfills the growth requirements of *H. influenzae*. It seems of interest to point out that all type b strains tested produced the type-specific substance in this medium.

Yeast autolysate alone does not permit maximal growth of *H. influenzae*. This conclusion is based on the following observations: (1) A larger inoculum is necessary in order to promote growth in 10 per cent yeast autolysate-saline solution than in 10 per cent yeast autolysate-brain heart infusion. In several experiments the respective inocula were 10^{-3} for yeast autolysate alone and less than 10^{-6} for yeast autolysate-brain heart infusion. (2) Growth in yeast autolysate-saline solution usually is somewhat less profuse and takes place more slowly than in yeast autolysate-brain heart infusion.

On the basis of the foregoing data yeast autolysate appears to be a suitable medium for the growth of *H. influenzae*, and it seems possible that this material may be used to advantage in cultural examinations. Preliminary to such studies, experiments were carried out on the growth-promoting properties of yeast autolysate in spinal fluid procured under aseptic conditions from patients free of meningitis. Both cell count and protein content were within normal limits. It could be shown that *H. influenzae* type b grows well in 10 per cent yeast autolysate-spinal fluid and produces the type-specific soluble substance. One strain was maintained in this medium for 19 serial transfers. That yeast autolysate, indeed, may be of aid in the diagnosis of meningitis caused by *H. influenzae* is suggested by the following observation: Identical amounts of spinal fluid (no microorganisms seen on film) from a patient with clinical meningitis were seeded on hemoglobin-proteose #3 agar, blood agar, and into 10 per cent yeast autolysate-brain heart infusion. After incubation at 37°C. for 18 hours profuse cloudiness was present in the yeast autolysate medium, whereas only minute colonies were seen on the solid culture media. Anti-*H. influenzae* type b serum produced specific precipitation and/or agglutination with the yeast autolysate culture.

Determination of the sensitivity to streptomycin of strains of *H. influenzae* isolated from patients prior to, and concurrent with, chemotherapy is of practical importance. It was decided, therefore, to investigate the possible usefulness of yeast autolysate as a culture medium for such tests. Thus far 12 strains have been studied in this medium, using streptomycin in

amounts of 1 and 10 μg./ml. The results of a representative experiment are summarized in Table 2.

It is evident that streptomycin prevents or inhibits the growth of the organism. Attention may be called to the fact that the size of the inoculum influences the degree of bacteriostasis. All 12 strains tested were inhibited by streptomycin in amounts ranging between 1 and 10 μg./ml. This finding corresponds closely to the results obtained on solid culture media: *H. influenzae* is inhibited by 5 μg./ml. of agar. Using yeast autolysate as the culture medium, the results may be obtained within 18 hours. Furthermore, it appears from the data presented above that such a test can be carried out even if only small numbers or organisms are available for the inoculation of the yeast autolysate medium.

TABLE 2

BACTERIOSTATIC EFFECTS OF STREPTOMYCIN UPON *H. influenzae* TYPE b IN 10 PER CENT YEAST AUTOLYSATE-BRAIN HEART INFUSION

Hours of observation	Streptomycin 10 μg./ml.	1 μg./ml.	0 μg./ml.
	Inoculum 0.2 ml. of undiluted culture		
18	−	++++	++++
24	−	++++	++++
48	−	++++	++++
	Inoculum 0.2 ml. of 1:100 diluted culture		
18	−	++++	++++
24	−	++++	++++
48	−	++++	++++
	Inoculum 0.2 ml. of 1:10,000 diluted culture		
18	−	+	++++
24	−	++	++++
48	−	++++	++++
	Inoculum 0.2 ml. of 1:1,000,000 diluted culture		
18	−	−	++++
24	−	−	++++
48	−	−	++++

− = no visible growth; + to ++++ = various degrees of visible growth.

The experiments presented revealed that yeast autolysate contains the essential growth factors required by *H. influenzae*. All strains tested thus far could be grown and maintained both in 10 per cent yeast autolysate-saline solution and in yeast autolysate-brain heart infusion. It is worthy of note that these culture media promote rapid multiplication of the organism and that the resulting growth causes grossly visible cloudiness. This observation seems to be of significance, since, as stated by Hoagland and his associates (*4*) in 1942, "turbidity produced by growth of *H. influenzae* is rarely great, even under optimum conditions." The question remains as to the nature of the X factor present in yeast autolysate. Studies are needed to determine whether this material contains a factor identical with, or similar to, the active component in hemin or whether some other substance such as catalase acts as X factor.

From a practical point of view the demonstration of the growth-promoting properties of yeast autolysate may find several applications. It seems quite possible that yeast autolysate infusion broth may be used to advantage for the growth of *H. influenzae*, particularly for the isolation of this organism from spinal fluid, blood, pus, etc. Preliminary studies indicate that growth may be obtained in the yeast autolysate medium more rapidly and with smaller inocula than on hemoglobin-proteose #3 agar. It is worthy of note that all type b strains grown in yeast autolysate produced the type-specific soluble substance. Studies are indicated, therefore, to determine quantitatively the amount of SSS formed in this culture medium. Such a study may yield information on the potential value of yeast autolysate cultures as immunizing antigens used in rabbits, a procedure which may be employed for the production of therapeutically effective antiserum. It has been shown, furthermore, that yeast autolysate-brain heart infusion can be used in tests designed to determine the streptomycin sensitivity of strains of *H. influenzae*.

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A Simple Method for Changing Units

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Students and research workers frequently experience difficulty in converting a quantity from one system of units to another. The following rule, which the writer has used in his classes for several years, eliminates the uncertainty in such computations by reducing them to a routine procedure.

Rule: *Write the quantity with its units in the first system. Give to each unit the coefficient 1. Substitute for each of these units its value in the second system. Reduce all numerical factors to a single coefficient, which is the numerical value of the quantity in the second system of units.*

Example: Given, the coefficient of thermal conductivity of glass:

$$k = 0.00250 \frac{\text{cal. cm.}}{\text{cm.}^2\ ^\circ\text{C. sec.}}.$$

Required, k in terms of B.t.u. per in. thickness per ft.^{2} per °F. per hr.

Applying the rule,

$$k = 0.00250 \frac{[1\ \text{cal.}]\,[1\ \text{cm.}]}{[1\ \text{cm.}^2]\,[1^\circ\text{C.}]\,[1\ \text{sec.}]}$$

$$= 0.00250 \frac{\left[\frac{1}{252}\ \text{B.t.u.}\right]\left[\frac{1}{2.54}\ \text{in.}\right]}{\left[\frac{1}{30.48}\ \text{ft.}\right]^2\left[\frac{9}{5}\ ^\circ\text{F.}\right]\left[\frac{1}{3{,}600}\ \text{hr.}\right]}$$

$$= 7.26 \frac{\text{B.t.u. in.}}{\text{ft.}^2\ ^\circ\text{F. hr.}}.$$