

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

The Kinetics of Gaseous Addition of Halogen Acids to Isobutene

BY G. B. KISTIAKOWSKY AND C. H. STAUFFER

In a recent paper¹ the thermal decomposition of tertiary butyl chloride into hydrogen chloride and isobutene has been shown to be a clean gaseous unimolecular reaction. The present communication deals with some measurements of the tertiary butyl bromide decomposition, and with the study of the reverse reactions. These latter are of considerable interest because work on liquid phase reactions of this type has led to the discovery of peculiar effects, due supposedly to the presence of oxygen or peroxides,² and because Maass³ has claimed that a discontinuity in reaction rates exists in the transition from the liquid to the gaseous state which cannot be explained by the concentration effect alone.

Equilibrium between halogen acids and olefinic hydrocarbons in the gaseous state has been investigated in the past infrequently. The work of Brunel⁴ on isobutene and hydrogen bromide will be discussed later. Wibaut, Rutgers and Diekmann⁵ reported an 84.4% yield of ethyl chloride at *ca.* 200° and atmospheric pressure, while Tilman⁶ finds about 4% decomposition of ethyl chloride around 120°. These studies have been made with the aid of catalysts. While they possibly cannot be regarded as very accurate determination of equilibria, they indicate, as will be seen from comparison with the data here presented, that the free energies of formation of alkyl halides are not greatly dependent either on the alkyl or on the halogen constituent of the molecule.

The study of the decomposition of tertiary butyl bromide using technique developed earlier for the chloride presented no new difficulties, but repeated experiments made to study the addition of hydrogen chloride and bromide to ethylene, pentene-2 and isobutene⁷ failed to discover any homogeneous reaction. This is in accord with the findings of Maass, but it does not mean that

the gaseous kinetics of these reactions are in any way "abnormal." As will be seen later, the activation energies of the association reactions with isobutene are fairly high, causing slow rates at lower temperatures, while at higher temperatures the equilibria are too unfavorable for a study at and below atmospheric pressure.

It thus became necessary to determine their kinetics by combining rate expressions for the decomposition with equilibria data; the latter were obtained with the aid of catalyzed reactions. This procedure suffers, of course, from increased errors, but the over-all accuracy is sufficient, we believe, to draw the most essential conclusions.

Experimental

The decomposition of *t*-butyl bromide and the equilibrium composition in both reactions were studied in a static system by pressure measurements. For the decomposition experiments the system was the same as described previously;¹ it was used also for the equilibria studies, but in this case the containers of the halogen hydrides were separated from the reaction flask by a glass valve, whereas between the isobutene and the *t*-butyl halide containers and the flask were stopcocks followed by a glass valve. This latter communicated also, through a third stopcock, with the pumping system.

The same treatments of the flask, even perhaps somewhat more protracted, were necessary to obtain a homogeneous decomposition of *t*-butyl bromide as were described in the case of *t*-butyl chloride.

For the equilibria studies flasks of varying catalytic activity were used at the several temperatures studied. For the lowest temperature a one-liter flask filled with 100 g. of glass wool (soft glass) was prepared; the others were filled with broken glass (Pyrex) of about pea size and with pieces of 10-mm. tubing, while for the highest temperatures an empty flask was found to give the most convenient rates. The equilibrium was usually indicated by a complete cessation of pressure changes, but in some cases a very slow, steady decrease of pressure was observed, due, no doubt, to a polymerization reaction of the olefin. In these runs the equilibrium pressure was extrapolated to zero time assuming a constant rate of polymerization. The whole correction was small.

According to Brunel,⁴ besides the *t*-butyl halide, considerable quantities of the isobutyl halide are formed at temperatures in the neighborhood of 270° (25% of isobutyl bromide), while at 100° their relative yield is negligible. If true, this finding would indicate that the heat of formation of the iso compound differs very considerably from that of the tertiary halide, while the free energies of their formation are nearly identical at 270°. Such a conclusion

(1) Brearley, Kistiakowsky and Stauffer, *THIS JOURNAL*, **58**, 43 (1936).

(2) Kharsch and Hinckley, *ibid.*, **56**, 1243 (1934).

(3) Maass, *ibid.*, **46**, 2664 (1924); **47**, 2883 (1925).

(4) Brunel, *ibid.*, **39**, 1978 (1917).

(5) Wibaut, Diekmann and Rutgers, *Rec. trav. chim.*, **47**, 479 (1928).

(6) Tilman, Thesis, Amsterdam, 1928.

(7) Hydrogen bromide and isobutene have a barely measurable homogeneous rate at 300°. About 2% reaction occurs in six minutes with little further change.

is not very plausible and Brunel's measurements were repeated avoiding his most probable source of error, a reaction between condensed isobutene and hydrogen bromide. The method consisted in passing a metered mixture of hydrogen chloride and isobutene through a flask packed with glass wool and maintained at 270°. The contact time was more than amply sufficient to establish equilibrium. After leaving the furnace the gases passed through several bubblers containing a 7.5% solution of sodium bicarbonate, to remove hydrogen chloride, then a calcium chloride tube and a trap at 0° in which the alkyl chlorides were collected; another trap, at -80°, collected the unreacted isobutene. About 13 cc. of butyl chlorides was obtained. This was distilled through a 3-ft. (91 cm.) Poddbielniak-type column with a 10:1 reflux ratio. Less than 0.5 cc. came over before 50.4° and the rest boiled at 50.6° until the pot was dry. The hold-up in the column amounted to 1.5 cc. It had a refractive index of n_D^{20} 1.3901, while the distillate had a (uniform) refractive index of 1.3839. If this entire difference is attributed to the presence of isobutyl chloride in the residues, its concentration in the initial mixture is found to be 7.8%. Considering all probable errors, such as the higher volatility of *t*-butyl chloride, the presence of polymers, etc., the real concentration is probably smaller. This experiment could not be repeated with hydrogen bromide because of rapid hydrolysis of *t*-butyl bromide by the bicarbonate solution, but we believe that essentially the same conditions prevail in that system also. Brunel's finding is accountable by a reaction between hydrogen bromide and isobutene when these were condensed to a liquid, which would probably give, since oxygen was not carefully excluded, mainly isobutyl bromide. In his experiments at 100°, on the other hand, only a little free hydrogen bromide remained upon establishment of gaseous equilibrium and thus the liquid phase reaction was insignificant.

Preparation of the Materials.—The *t*-butyl bromide was obtained from Eastman Kodak Co. and was purified by a slow crystallization with stirring. When approximately half of the material was frozen, the crystals were separated from the liquid, and used for the kinetic experiments. No difference in results was observable between different samples. The *t*-butyl chloride was made as described previously.¹

The isobutene was part of the material prepared in this Laboratory for the calorimetric study of its heat of hydrogenation⁸ and was used without further purification.

Hydrogen chloride was prepared by dropping concd. c. p. sulfuric acid on sodium chloride and concd. hydrochloric acid solution. It was dried over sulfuric acid, liquefied and distilled once from trap to trap *in vacuo*. The middle third was used.

Hydrogen bromide was prepared by dropping bromine on naphthalene. The evolved gases were led over naphthalene and copper turnings, to remove unreacted bromine. Hydrogen bromide was liquefied and distilled from trap to trap once. The middle third was used.

Results and Interpretation.—A summary of the rate constants and the experimental data for

(8) Kistiakowsky, Ruhoř, Smith and Vaughan, *THIS JOURNAL*, **57**, 876 (1935).

the thermal decomposition of the tertiary butyl bromide will be found in Table I. Run 95p was carried out in a packed flask having ten times the surface volume ratio of the unpacked flask. The packed flask had been given nine treatments to deactivate the glass surface. Each treatment consisted in allowing about 200 mm. of *t*-butyl bromide to decompose in the flask at a temperature of 500° for from five to ten hours. The comparison indicates quite clearly the homogeneity of the reaction.

TABLE I
DECOMPOSITION OF *t*-BUTYL BROMIDE

Run	T, °K	P, mm.	$k \times 10^4$, sec. ⁻¹
87	508.9	97	0.836
95p	509.9	101	1.07
88	510.2	96	1.01
84	526.1	93	3.41
82	540.5	101	9.40
85	559.3	95	32.5
90	562.5	48	40.0
83	562.7	95	42.0
86	564.8	95	44.0

In Tables II and III will be found the equilibrium constants for the chloride and bromide, respectively. Runs whose numbers include an (a) are those in which the equilibrium was approached from the side of addition, while the letter (d) refers to an approach through the decomposition reaction. Those containing (ad) refer to runs in which the tertiary bromide was first decomposed at a rate too rapid to get an accurate initial pressure, and then the reaction was forced back by introducing a measured amount of hydrogen bromide and following the pressure until the equilibrium was again established. The usual plot of the decomposition rate constant of the bromide gives a good straight line and leads to an activation energy of 40,500 =

TABLE II
EQUILIBRIUM BETWEEN HYDROGEN CHLORIDE, ISOBUTENE AND *t*-BUTYL CHLORIDE

Run	T, °K.	$P_{\text{equil.}}$, mm.	K , atm. ⁻¹
1d	361.3	263.5	284
2d	364.9	265.8	234
3d	368.0	280.0	194
4d	377.4	287.5	110
5d	380.4	290.8	92.0
6d	381.4	209.6	80.1
7d	415.0	228.9	14.1
8d	415.2	288.0	13.7
9d	421.3	340.6	10.6
10a	509.5	414.9	0.284
11a	510.4	484.6	.256

TABLE III
EQUILIBRIUM BETWEEN HYDROGEN BROMIDE, ISOBUTENE
AND *t*-BUTYL BROMIDE

Run	T, °K.	P _{equil.} , mm.	K, atm. ⁻¹
12ad	375.0	297.7	440
13ad	380.8	278.8	385
14d	382.8	157.4	443
15ad	384.8	306.5	410
16d	386.3	190.0	332
17d	400.2	119.2	130
18d	401.7	117.7	142
19d	402.0	115.8	129
20d	405.9	116.6	118
21d	409.2	119.5	88.4
22d	410.3	132.3	97.5
23d	410.7	163.0	82.0
24d	426.0	133.9	37.4
25a	443.2	127.1	11.8
26a	445.9	132.6	9.94
27d	471.1	163.2	4.21
28d	473.6	161.2	4.14
29d	473.6	164.1	3.94
30d	475.6	226.9	3.25
31d	475.8	165.9	3.49
32d	477.8	228.1	3.07
33d	479.4	228.2	3.05
34a	482.9	251.5	2.45
35d	508.5	189.4	1.32
36a	514.2	303.8	0.724
37a	515.1	301.8	.821
38a	531.7	218.1	.438

1000 cal. Using this value, the expression for the rate constant can be cast into the form

$$(\text{Bromide}) k_d = 10^{18.3 \pm 0.4} e^{-(40,500 \pm 1000)/RT} \text{ sec.}^{-1}$$

To be compared with it is the expression for the decomposition of the chloride:¹

$$(\text{Chloride}) k_d = 10^{13.9 \pm 0.7} e^{-(46,000 \pm 1900)/RT} \text{ sec.}^{-1}$$

It is apparent that while the activation energies

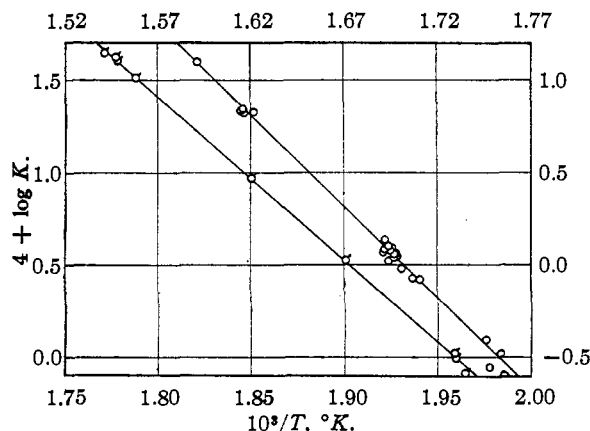


Fig. 1.—Plot of the reciprocal of the absolute temperature against the logarithm of the unimolecular rate constant. Upper curve refers to the decomposition of *t*-butyl chloride, and the lower curve to *t*-butyl bromide.

of the two reactions are markedly different (see Fig. 1), the *A* factors, within experimental uncertainties, are identical. The lower activation energy of the bromide is in good accord with the lesser stability of this compound as is known from general chemical experience.

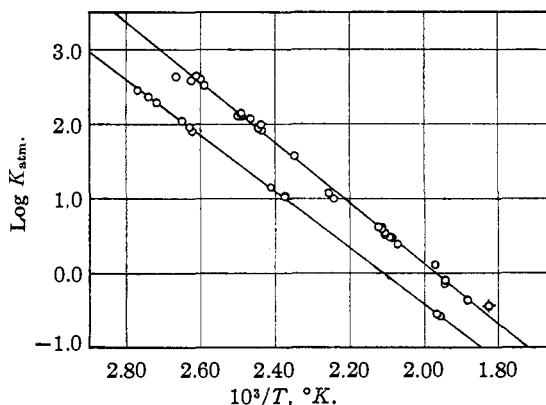


Fig. 2.—Plot of the reciprocal of the absolute temperature against the logarithm of the equilibrium constants expressed in atmospheres. The upper curve refers to the HBr, isobutane, and *t*-butyl bromide equilibrium, and the lower curve to the HCl, isobutane, and *t*-butyl chloride equilibrium. The crossed circle is a value found by Brunel.

The logarithmic plots of the two equilibrium constants will be found in Fig. 2 together with the values given by Brunel.⁴ Both equilibria give good straight lines and the expressions for the constants can be cast into the forms

$$\begin{aligned} (\text{Chloride}) \log K &= (17,100/4.58T) - 7.89 \\ \Delta F^\circ &= 17,100 + 36.1T \text{ (atmospheres standard)} \end{aligned}$$

with an uncertainty of *ca.* 500 calories in the heat of reaction;

$$\begin{aligned} (\text{Bromide}) \log K &= (18,900/4.58T) - 8.12 \\ \Delta F^\circ &= -18,900 + 37.2T \end{aligned}$$

with an uncertainty of *ca.* 900 calories in the heat of reaction. The difference of the two heats of reaction exceeds the probable error, even when the uncertainty caused by the possible formation of isobutyl halides is included. As stated before, Brunel's finding of a large amount of iso compound at 270° could not be substantiated and a plausible explanation of his experimental data has been offered. A correction for 8% iso chloride found at 270° (and a negligible amount at low temperatures) would change the heat of reaction only by some 200 cal./mole and can thus be disregarded in the following calculations.

Changing the equilibrium constants to mole/cc.

as units, converting the heats of reactions to constant volume and utilizing the relation

$$K = k_{\text{ass.}}/k_{\text{dec.}}$$

the following expressions are obtained for the association rate constants

$$\text{(Chloride)} \quad k = 10^{11.0 \pm 1.0} e^{-(28,800 \pm 2400)/RT} \text{ cc./mole sec.}$$

$$\text{(Bromide)} \quad k = 10^{10.2 \pm 0.7} e^{-(22,600 \pm 1900)/RT} \text{ cc./mole sec.}$$

As before, the A factors can be considered as identical within experimental errors, while the activation energies are decidedly different. This difference is even more marked than in the decomposition reactions.

The A factors of these reactions are considerably smaller than the usual kinetic collision factors, but this is to be expected in association reactions of large molecules and the best method to investigate whether they are "normal" is to compare them to the theoretical expressions for the rates of such reactions, as developed recently in several publications. Use will be made of the Eyring⁹ notation and concept of the activated complex but no attempt will be made to calculate the potential energy surface of the reaction. It will be assumed instead that the activated complex is identical in size and shape with the final molecule (*t*-butyl chloride), an assumption made by Rice and Gershinowitz¹⁰ in their "exact orientation" theory. An estimate of the vibrational frequencies of the activated complex being uncertain, to say the least, these frequencies will be regarded as unknown and to be deduced from a comparison of the calculated and measured rate constants.

Two possibilities offer themselves for calculation. One may regard that CH_3 group, in the activated complex, which becomes $=\text{CH}_2$ group in isobutene as possessing free rotation. Then the activated complex is identical with *t*-butyl chloride in all degrees of freedom, but one, and the calculation conforms entirely to the "exact orientation" theory. Or, one may suppose that free rotation does not exist in the activated complex and that instead a torsional vibration (of low frequency) is present. The two methods of calculation lead to formally different equations. However, no decision is possible between them because numerically very similar results are obtained in both cases. This is due to the circumstance that the partition function of internal rotation of a CH_3 group (one degree of freedom) is

almost equal numerically to the extra symmetry factor to be assigned to the activated complex when it is assumed to have free internal rotation of that group. If the activated complex has free rotation of all three (equivalent) methyl groups, its symmetry factor is taken at 3^4 ; if free rotation exists in only the non-reacting methyl groups, the factor is 3^2 . The symmetry factor of the non-activated *t*-butyl chloride is 3^4 , that of isobutene 18. The internal rotational partition function of CH_3 is calculated to be equal to 7.2 at 500°K . The other numerical data used are:

The product of the moments of inertia of isobutene is $6.6 \times 10^{-115} \text{ g.}^3 \text{ cm.}^6$. The product of the moments of inertia of *t*-butyl chloride is $58 \times 10^{-115} \text{ g.}^3 \text{ cm.}^6$. The moment of inertia of hydrogen chloride is $2.6 \times 10^{-40} \text{ g. cm.}^2$. The former two have been calculated using Pauling's internuclear distances and tetrahedral angles. Using the internal free rotation model we find for the association reaction rate constant

$$k_{\text{ass.}} = 1.5 \times 10^9 \frac{F^*}{F_1 F_2} \frac{500}{T} e^{-(E_0 - RT)/RT} \text{ cc. mole}^{-1} \text{ sec.}^{-1} \quad (1)$$

The F 's stand here for the vibrational partition functions, F^* containing three more of them than F_1 and F_2 together. The value before the exponential cannot be compared directly with the experimental figure because of differences in the activation energy. The following procedure which has been adopted to correct E_0 to the temperature of the experiments is not free from objections but it seems to us to be practically the most reasonable one. The rate constant for the decomposition reaction is calculated to

$$k_{\text{dec.}} = 2.8 \times 10^{13} \frac{F^*}{F_3} \frac{T}{500} e^{-(E_0 + RT)/RT} \text{ sec.}^{-1} \quad (2)$$

where F^* contains one less vibration than F_3 . $E_0 + RT$ is the activation energy at the temperature of the experiments unless the vibrational heat capacities of the associated molecule and the activated complex are different. Comparing the numerical factor in (2) with the experimental values, one finds that they are practically identical for the *t*-bromide, while that of the *t*-chloride is about three times larger. This we are inclined to ascribe to experimental errors, in accord with the "exact orientation" theory which does not assume any loosening of the molecule on transition to the activated state and hence prescribes essentially identical vibrational partition functions for the reactant molecule and the activated

(9) Eyring, *J. Chem. Phys.*, **3**, 107 (1935).

(10) Rice and Gershinowitz, *ibid.*, **3**, 479 (1935).

complex. Because the equilibria data can be regarded as quite accurate, the experimental association rate A -factor of the chloride must also be reduced by a factor three to make everything self-consistent. The ratio of the experimental value to the above calculated one is then equal to about nine. Assuming now that the difference between E_0 and the experimental activation energy is due to the T factor in equation (1) and the contribution of the three extra vibrations in F^* , one finds by trial that a factor four for the product of the three extra vibrational partition functions in F^* gives the correct answer. Hence, the average frequency of the new vibrations in the activated complex is *ca.* 340 cm.^{-1} .

For the model of the activated complex, in which the CH_2 group of the butene has not acquired free rotation, one finds

$$k_{\text{ass.}} = 1.1 \times 10^9 \frac{F^*}{F_1 F_2} \left(\frac{500}{T}\right)^{3/2} e^{-(E_0 - 3/2 RT)/RT} \text{ cc. mole}^{-1} \text{ sec.}^{-1} \quad (3)$$

and

$$k_{\text{dec.}} = 2.2 \times 10^{13} \frac{F^*}{F^{\ddagger}} \left(\frac{T}{500}\right)^{1/2} e^{-(E_0 + 1/2 RT)/RT} \text{ sec.}^{-1} \quad (4)$$

where F^* contains four more vibrations than F_1 and F_2 together and the same number as F_3 . Proceeding much in the same manner as outlined before, one arrives at an average frequency of vibrations formed in the association reaction of about 450 cm.^{-1} . In view of the experimental errors and the many approximations made in calculations it is impossible to make a selection between these two sets of data. Both are quite reasonable although, as in the case of Diels-Alder association reactions,¹¹ somewhat higher frequencies would have been even more acceptable. In any case it should be clear from the preceding calculations that the association of halogen hydrides and isobutene in the gas phase proceeds according to entirely normal kinetics, calculable, in the order of magnitude at least, by the statistical theory of reaction rates. Some details of the calculations and some experimental data are not as perfect as could be desired but the main purpose of this investigation—a demonstration that the kinetics of these associations are “normal”—has been achieved. They cannot be observed directly merely because of coöperation of a high energy of activation and an unfavorable equilibrium. This applies *a fortiori* to the formation of secondary and primary halides because their

energies of activation are probably still higher. This may be deduced tentatively from the generalization that the stability of primary and secondary halides is greater than that of the tertiary ones,¹² coupled with the not greatly different free energy change in the reaction.

Some remarks concerning association reactions of halogen acids and ethylenic hydrocarbons in liquid media are, perhaps, in order here. Present work leaves no doubt that these reactions at and near room temperature proceed via some mechanism quite different kinetically from the one here discussed. This is inevitable since, even allowing for concentration changes, one finds vanishingly small reaction rates at room temperature, with the expressions here given, whereas rapid rates have been observed in several widely different solvents.^{2,5} This latter circumstance would make it very improbable that the fast rate is due to some specific effect of solvent on the activity of the reactants, or on that of the activated complex. Hence, the most plausible mechanism accounting for the reaction rate in liquids is a chain mechanism. In the light of such interpretation the findings of Kharasch,² on the action of oxygen, may find a reasonable explanation, although on the whole the effect is puzzling. In this connection it may be added also that in the gas phase reaction the addition of small amounts of oxygen does nothing but accelerate the wall reaction.

Summary

The rate of gaseous thermal decomposition of *t*-butyl bromide has been investigated in the temperature region of 509–564°K., and it was found to be a homogeneous unimolecular reaction.

The reaction rate constant was found to be

$$k = 10^{13.3 \pm 0.4} e^{-(40,500 \pm 1000)/RT} \text{ sec.}^{-1}$$

The equilibrium of *t*-butyl bromide and *t*-butyl chloride with isobutene and the respective halogen acid was investigated in the temperature region 361–533°K.

The heats of reaction at constant pressure for the associations were found to be $-17,100 \pm 500$ cal., and $-18,900 \pm 900$ cal. per mole for the formation of the chloride and the bromide, respectively.

The free energy changes of the reactions were found to be expressed in the form

$$\begin{aligned} (\text{Chloride}) \quad \Delta F^\circ &= -17,100 + 36.1T \\ &\quad \text{(atmospheres standard)} \\ (\text{Bromide}) \quad \Delta F^\circ &= -18,900 + 37.2T \end{aligned}$$

(11) Kistiakowsky and Lacher, *THIS JOURNAL*, **58**, 123 (1936).

(12) *Cf.* Vernon and Daniels, *ibid.*, **55**, 922 (1933).

The rate equations of the association reactions were calculated from these data and found to be:

$$\begin{aligned} (t\text{-Chloride}) \quad k &= 10^{11.0 \pm 1.0} e^{-(28,800 \pm 2400)/RT} \\ (t\text{-Bromide}) \quad k &= 10^{10.2 \pm 0.7} e^{-(22,500 \pm 1900)/RT} \end{aligned}$$

The units are cc. moles⁻¹ sec.⁻¹.

The isomerization of *t*-butyl chloride to isobutyl

chloride at 270° was investigated, and found to be less than 7.8% at equilibrium.

The statistical mechanical explanation of slow reaction rates in the form developed by Eyring was applied, and reasonable agreement was found with the theory.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND THE DEPARTMENT OF HYGIENE AND BACTERIOLOGY, UNIVERSITY OF CHICAGO]

Some Chemical Properties of an Essential Growth Factor for Pathogenic Bacteria

BY FELIX SAUNDERS, I. I. FINKLE, LEON STERNFELD AND STEWART A. KOSER

Many bacteria which develop quite readily in a meat-infusion culture-medium are unable to grow in a simple synthetic medium composed of several amino acids, dextrose, and inorganic salts. This observation is particularly true of many of the pathogenic types and has given rise to considerable speculation concerning the nature of the substances in meat infusions which appear to be necessary for development. Several attempts have been made to fractionate meat infusions or other similar mediums in the hope of gaining an insight into the nature of the factors essential for growth of the more exacting bacteria.¹ The same method of study has also been applied to yeasts.²

In previous work of our own³ it was found that a growth-stimulating fraction could be removed from veal infusion by adsorption on charcoal and subsequent elution with hot ethanol or acetone. When this fraction was added to a simple synthetic medium⁴ it rendered the medium suitable for the growth of some, though not all, of the more exacting pathogenic bacteria. In certain instances very small amounts of this added factor were sufficient to permit development in a synthetic medium in which the organisms ordinarily refused

to grow. The results of a representative experiment with three microorganisms belonging to different biological groups are given in Table I.

The luxuriance of growth, varying from very light to heavy turbidity, is shown by the number of + signs. Absence of visible turbidity is shown by 0. An occasional doubtful reading is recorded ?. Readings were also made after seven and fourteen days, but have been omitted from the table as in most instances they showed no change.

The strain of *C. diphtheriae* used was a culture of "Park 8," that of the Sonne dysentery bacillus was no. 268 of the British Type Collection, and the strain of *Staph. albus* was from our laboratory stock collection.

Here it is seen that two of the three microorganisms refused to develop in the synthetic medium alone. The third organism, the so-called Sonne type of dysentery bacillus, grew very sparsely in the synthetic medium. When the fraction from veal infusion was added all of the organisms developed very well and produced a heavy growth in a few days. Relatively small amounts of this added material were effective and it is evident that something quite essential for multiplication has been added.

Is the added material a foodstuff or something in the nature of an accessory growth factor? It is obvious that the fraction from veal infusion must consist of a mixture of compounds and one would expect some food materials to be carried over along with any accessory factor which might be present. The results with the inorganic salt solution shown in Table I indicate that only a negligible amount of actual food material was contained

(1) (a) Thjötta and Avery, *J. Exptl. Med.*, **34**, 97, 455 (1921); (b) Mueller, *J. Bact.*, **7**, 309 (1922); (c) Mueller, *et al.*, *ibid.*, **25**, 509 (1933); **30**, 513 (1935); (d) Whitehead, *Biochem. J.*, **17**, 742 (1923); **18**, 829 (1924); (e) Knight and Fildes, *Brit. J. Exptl. Path.*, **14**, 112 (1933); (f) Sahyun *et al.*, *J. Infectious Diseases*, **56**, 28 (1936).

(2) See especially Williams, *et al.*, *THIS JOURNAL*, **55**, 2912 (1933); *Proc. Soc. Exptl. Biol. Med.*, **32**, 473 (1934); Miller, Eastcott and Maconachie, *THIS JOURNAL*, **55**, 1502 (1933); *Kögl. Ber.*, **68**, 16 (1928).

(3) Koser and Saunders, *J. Infectious Diseases*, **56**, 305 (1935).

(4) The synthetic medium consisted of 1.4 g. Na₂HPO₄, 1.0 g. KH₂PO₄, 2.0 g. NaCl, 0.1 g. MgSO₄, 3.0 g. asparagine, 0.1 g. cystine, 0.2 g. tryptophane, and 2.0 g. dextrose in 1 liter of distilled water. When an inorganic salt solution was desired for control purposes the asparagine, cystine, tryptophane and dextrose were omitted.