internal rotation of simple molecules closely related to the polypeptide chain.[8]

(8) See also S. Mizushima, T. Shimanouchi, *et al.*, THIS JOURNAL, **73**, 1330 (1950); **74**, 270 (1952); *Nature*, **169**, 1058 (1952).

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HYDROGEN TRANSFER REACTIONS ACCOMPANYING THE COBALT CATALYZED SYNTHESIS FROM ACETYLENE, CARBON MONOXIDE AND METHANOL

Sir:

Acetylene reacts with carbon monoxide and methanol in presence of a cobalt catalyst and it is known that in this reaction a mixture of esters can be obtained.[1] Using a dissolved cobalt catalyst, *e.g.*, $(Co(CO)_4)_2$ the reaction proceeds much more rapidly and no metallic cobalt is found in the reaction products.

Acting at very low temperature (90–110°) and at high CO pressure (200–300 atm.) in presence of 2% dissolved $[Co(CO)_4]_2$ as catalyst the following products are identified, the yield being dependent primarily on the concentration of the acetylene:

	Product	Yield, g./100 g. C_2H_2	Notes
1	Methyl acrylate	14–24	Identification by reaction with CH_2N_2[2]
2	Cyclopentanone	Traces	2,4-Dinitrophenylhydrazone m.p. 146°[3] mix. m.p. with an authentic sample, 146°
3	Δ^2-Cyclopentenone-1	2–10	B.p. (760 mm.) 154–155°; $n^{20}D$ 1.4787; U.V. spectrum[4] λ = 309 mμ, log ϵ = 1.50; 2,4-dinitrophenylhydrazone m.p. 165–166°,[5] *anal.* N: found 21.8; calcd. for $C_{11}H_{10}O_4N_4$: 21.37
4	Dimethyl fumurate	1–14	M.p. 101.2–101.4°; mixture m.p. with an authentic sample, 101.2°
5	Dimethyl succinate	70–250	M.p. 19°, $n^{20}D$ 1.4484
6	Trimethyl ethanetricarboxylate	35–50	M.p. 34.5°; *anal.* found C, 47.19; H, 5.89; calcd. for $C_8H_{12}O_6$, C, 47.058; H, 5.882
7	Dimethyl γ-ketopimelate	40–60	M.p. 56°; *anal.* found: C, 53.3; H, 6.85; calcd. for $C_9H_{14}O_5$: C, 53.46; H, 6.93

The formulas of the products 1 and 5 are in agreement with the addition of one or two molecules of carbon monoxide and methanol to one molecule of acetylene.

On the contrary the composition of the other products does not correspond to the sum of molecules of acetylene, carbon monoxide and hydrogen but shows a higher or lower hydrogen content. Therefore any solid catalyst being absent the products 2, 4, 6, 7, must arise from some homogeneous hydrogen transfer reaction like the one observed[6] in the synthesis of esters or amides from olefins, carbon monoxide and alcohols or amines.

(1) G. Natta and P. Pino, Swiss Patent Ges. N. 46197 (June 24, 1949); *La Chimica e l'Industria*, **31**, 249 (1949).
(2) *Ber.*, **33**, 3595 (1901).
(3) THIS JOURNAL, **57**, 758 (1935).
(4) *Ibid.*, **74**, 514 (1952).
(5) *Ann.*, **539**, 207 (1939).
(6) G. Natta, P. Pino and R. Ercoli, THIS JOURNAL, **74**, 4496 (1952).

The hydrogen necessary for the synthesis of the cycloketones and γ-ketopimelic acid dimethyl ester, does not seem to be supplied by the dehydrogenation of methanol because no appreciable amounts of formaldehyde, or its derivatives, has been detected.

Also the formation of hydrogen by reaction between carbon monoxide and water cannot proceed to a large extent because anhydrous reagents were used, and no dehydration products of the reactants (for example, dimethyl ether) has been found in the liquid or gaseous reaction products.

Presently it seems more probable that the succinic acid dimethyl ester acts as hydrogen donor the first dehydrogenation product being the fumaric acid dimethyl ester.

Moreover, only a small amount of the last compound is detected in the reaction products, the most part being transformed in ethanetricarboxylic acid trimethyl ester, by reaction with carbon monoxide and methanol.

The very low temperature at which the synthesis of esters and ketoesters seems to take place,[7] from the intermediate olefinic compounds carbon monoxide and methyl alcohol, is not surprising if we consider the hydrogen transfer reactions as typical chain reactions.

We can conclude that hydrogen transfer reactions take place very easily with cobalt catalysts in presence of high carbon monoxide pressure. The cobalt catalysts probably act as hydrogen carriers according with the equilibrium

$$[Co(CO)_4]_2 + H_2 \rightleftarrows 2[Co(CO)_4]H$$

which can occur at high carbon monoxide pressure in presence of movable hydrogen atoms.

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(7) G. Natta, P. Pino and E. Mantica, *Gazz. Chim. Ital.*, **80**, 650 (1950); *La Chimica e l'Industria*, **32**, 201 (1950).

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THE MECHANISM OF VIRUS ATTACHMENT TO HOST CELLS. III[1]

Sir:

The chemical basis of virus attachment to its host cell has not previously been elucidated. Earlier studies from this laboratory have shown that the first phase of invasion of a host cell by a bacterial virus consists in establishment of strong electrostatic bonds between sites on the two surfaces.[2,3] Some of the evidence on which these conclusions were based is as follows: The initial attachment is exceedingly rapid, being diffusion-limited; its rate is constant between 0 and 37°; it can be readily reversed by appropriate changes of the ionic constituents of the medium; and its

(1) This work was supported by Research Contract No. AT-(29-1)-787 with the Division of Biology and Medicine, U. S. Atomic Energy Commission.
(2) T. T. Puck, A. Garen and J. Cline, *J. Exp. Med.*, **93**, 65 (1951).
(3) A. Garen and T. T. Puck, *ibid.*, **94**, 177 (1951).

characteristics are duplicated with considerable faithfulness when the bacteriophage is allowed to attach to cationic ion-exchangers. Other experiments, including analysis of the electrical mobility of both virus and host-cell[4,5]; demonstration of the specific requirement for cations in order to achieve virus-cell union[2]; and the proof that such cations bind to both virus and cell,[3,6,7] have shown that a preponderance of negative charge exists on the surfaces of virus and host. This must be neutralized before attachment can occur.

The experiments reported here were undertaken in an effort to identify the specific chemical groupings responsible for the bond formation constituting primary attachment. Virus and host cells were treated with reagents which block various reactive groups, and the attachment characteristics of these chemically modified entities were then studied. Virus-cell attachment was measured by the standard procedures described earlier.[2] In those experiments in which the reagent inactivated the virus, P^{32}-labelled virus was employed. By measuring the radioactivity of the supernatants from suspensions in which modified virus had been mixed with host cells, it was possible to determine whether the primary attachment reaction had been altered, regardless of any effect on the multiplication of the virus. Whenever a reagent consisted of several components, control tests were performed demonstrating that none of the constituents individually was responsible for the observed effects on attachment.

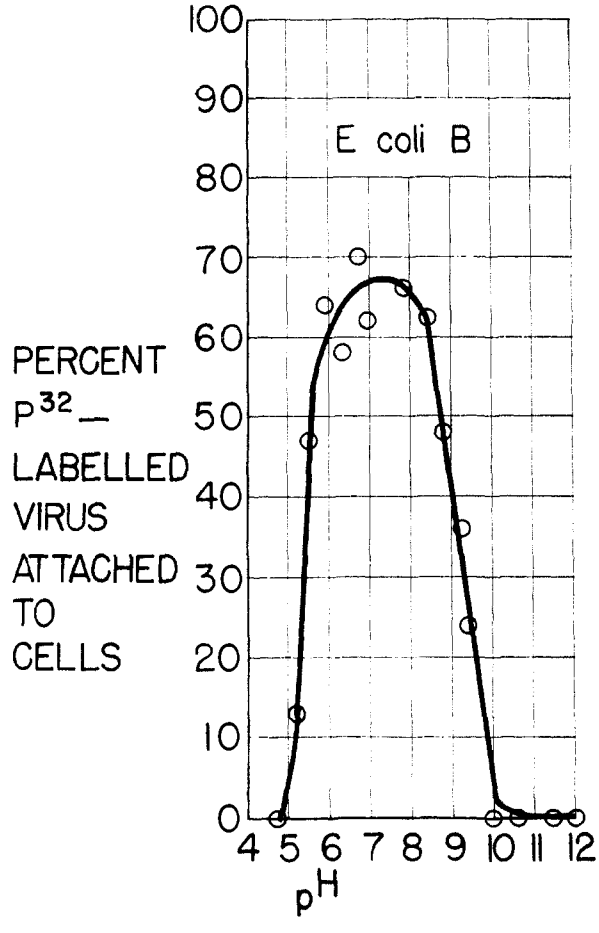

Fig. 1.—Experiments with *E. Coli. B:* values which differed by less than 0.1 *p*H unit have been averaged. Values which fell within the range −10% to +10% have been plotted as 0%, since the precision of the measurements is estimated to be ±10%.

Effect of *p*H.—The chemical groups whose involvement would seem most probable because of their ability to form ionic bonds are the following: carboxyl, amino and substituted amino, phosphoric acid, sulfhydryl, and phenolic-hydroxyl. Because of the wide differences in *pK* values of these groups, a study of the effect of *p*H on this bond formation was carried out. The attachment of T2 virus labelled with P^{32} to its host, *E. coli* B, is shown in Fig. 1. The attachment is reversibly inhibited by H^+ ion, and approaches zero at *p*H 4.8. This *p*H dependence rules out the possibility that bond formation is due primarily to phosphoric acid, sulfhydryl, or phenolic-hydroxyl groups because of the incompatibility of their *pK* values with the points of maximal *p*H effect. On the basic side, virus attachment falls to zero at *p*H 10. The shape of this *p*H curve suggests that carboxyl and possibly amino (or substituted amino) groups are taking part in this reaction, since specific attachment falls to zero at *p*H's beyond the limit at which these two groups ionize when incorporated in protein.

Treatment of Host Cells with Group-Specific Reagents.—Actively growing cells were treated for 30–50 minutes with the reagents indicated in Table I, at a temperature of 37° (except for nitrous acid and acetic anhydride which were used at 0°C.), and at *p*H 6.5–7.5 unless otherwise noted. The cells were then centrifuged, washed free of excess reagent, and resuspended in buffered salt solutions appropriate to the attachment of either

TABLE I

BINDING OF BACTERIAL VIRUSES T1 AND T2 TO CHEMICALLY MODIFIED HOST CELLS

Reagent used to modify cells	Virus attachment to modified cells: T2 Virus[a]	T1 Virus[a]
Carboxyl reagents		
Propylene oxide (satd.)	0	+
CH_3OH (80%) + HCl (0.1 *M*)	0	+++
Amino reagents		
Nitrous acid (0.5 *M*, *p*H 4.0)	++++	0
Formaldehyde (1.2 *M*)	++++	+
Acetic anhydride (0.3 *M*)	+++	++
Other reagents		
Na *p*-chloromercuribenzoate (5×10^{-4} *M*)	++++	++++
I_2 (5×10^{-3} *M*) + KI (0.5 *M*)	++++	++++
HCl (0.1 *M*)	++++	++++
CH_3OH (80%)	++++	++++

[a] Legend: 0 signified 0–30% virus attachment (compared with untreated cells); + signifies 30–45%; ++, 45–60%; +++, 60–80%; and ++++, 80–100%.

(4) F. W. Putnam, *Science*, **111**, 481 (1950).

(5) H. A. Abramson, L. S. Moyer and M. H. Gorin, "Electrophoresis of Proteins and the Chemistry of Cell Surfaces," Reinhold Publishing Corp., New York, N. Y., 1942, Chap. 14.

(6) J. Cleveland and T. T. Puck, forthcoming publication.

(7) W. Ruegamer, forthcoming publication.

T1 or T2 virus.[2] Under these conditions, untreated cells bound 80–100% of either virus. Each of the reagents listed caused complete loss of viability of the bacterial population, indicating that reaction with cellular structures had indeed taken place.

From the data in Table I, attachment of T2 virus appears to demand intact cellular carboxyl groups, but is independent of the integrity of amino groups on the cell surface. T1 virus, on the other hand, seems to require functional amino groups on its host and possibly carboxyl groups as well. Microscopic examination of cells treated with these reagents verified that cellular morphology and staining characteristics had remained intact. Hence, the failure of virus attachment cannot have been due to complete disintegration of the cells under the influence of the reagent. The ineffectiveness of sulfhydryl-blocking reagents like *p*-chloromercuribenzoate indicates that cellular sulfhydryl groups play no significant role in the primary union of these bacteriophages to their host.

These experiments must be interpreted in light of the reservations emphasized by the protein chemists who have studied group specificities of these compounds when applied to biological materials.[8] Thus, although the conditions employed were those which furnish maximal specificity, some secondary reactions like that of propylene oxide with amino groups could have occurred. However, there is good agreement within each group of cell reagents in their effect on virus attachment. Further, the fact that cells lose their ability to bind T2 virus only when treated with carboxyl-blocking reagents, but not when exposed to amino or sulfhydryl reagents, or to a strong acid or oxidizing agent, constitutes evidence of the chemical specificity associated with loss of attachment sites. Finally, the differential effect of amino reagents in depressing attachment of T1 virus but not of T2 is evidence that these effects result from the blocking of specific chemical groupings on the cell surface, rather than from random disorganization of cell structures.

These results also furnish a chemical basis capable of explaining the biological specificity of these viruses. Mutants of the original host cell exist which are completely resistant to attachment by only one of these viruses, but sensitive to the other. This differential sensitivity may be due to differences in the number and distribution of amino and carboxyl groups on the surfaces of the different cell mutants.

While the evidence that intact cellular carboxyl groups are necessary for attachment of T2 virus to its host does not prove that carboxyl bonding between the two surfaces occurs, any other interpretation seems unlikely. The physico-chemical evidence previously referred to indicates an ionic binding. Moreover, if attachment were accomplished only through secondary electrostatic forces like H-bond formation and dipolar and Van der Waals interactions, esterification of the carboxyl groups should promote rather than hinder cell attachment, because the repulsive force between virus and cell due to the excess negative charges on each body would be decreased. These considerations do not exclude participation of the weaker multipolar and Van der Waals forces in the union of virus and host. Such interactions undoubtedly contribute to the total energy of binding.

Treatment of Virus with Group-Specific Reagents.—Preliminary experiments were performed in which purified radioactive T2 virus was treated with the reagents listed in Table I. These experiments suggest that the amino and carboxyl groups of the virus surface also take part in the primary attachment reaction.

Summary.—The *p*H dependence of the attachment of T2 bacteriophage to its host cell suggests that ionization of carboxyl and amino groups is required for this reaction. Treatment of cells with carboxyl-blocking reagents suppresses their ability to bind the virus. Treatment of cells with a strong acid, an oxidizing agent, or with reagents which block amino and sulfhydryl groups, had no effect on their ability to bind T2. It may be concluded that cellular carboxyl groups participate in this reaction.

Cells treated with amino-blocking reagents lose most of their ability to bind T1 virus even though their affinity for T2 is unchanged. Thus virus-host specificity seems to be associated with the amino and carboxyl groups on the cell surface.

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(8) See, for example, H. S. Olcott and H. L. Fraenkel-Conrat, *Chem. Revs.*, **41**, 151 (1947); R. M. Herriott, *Advances in Protein Chem.*, **3**, 169 (1947); F. W. Putnam, in H. Neurath and K. Bailey, "The Proteins," Academic Press, Inc., New York, N. Y., in press, 1952.