APPENDIX

Amplifying Comments

Sugar Metabolism.

The highly efficient "smokeless" oxidation of sugar that leaves no traces of its existence by way of isolatable intermediaries, has been claimed by a number of brilliant biochemists. We believe it is the normal process and the others that are impeded enough to yield intermediaries are alternative paths provided to accommodate the need for vital energy production under the hindered circumstances that exist, such as anoxia, and injury. Isolation for study and the hindrances of the Warburg Chamber exemplify. In cancer a different hindrance exists. One resultant pathway has been ingeniously outlined by Krebs, but it has been erroneously accepted as the major process.

There are phenomena that suggest the "smokeless" process is one that is carried by a peroxide free radical, and the set-up may be as follows, but there is no real proof that the process is such. There is a strong confirmatory suggestion however in the competition offered by the amino groups of the oft used antibiotics, *which cause memory lapses,* periods of *suspended consciousness and the like,* that give rise to so many serious accidents. We therefore suggest that the F.M. especially of nervous tissue is provided with an activated amine group that condenses with the carbonyl group of fructose to form an azomethine bond, and that by an Amadori rearrangement this double bond passes to between C 2 and C 3. This of course mobilizes the hydrogen atom of C 4, and makes it subject to removal with free radical production, and in the presence of adequate oxygen, to peroxide free radical formation that carries the burning of the sugar to carbon dioxide and water. In the *absence of adequate oxygen* the sugar would split at this point to form lactic acid, or pyruvic acid. Catalysts that are required to facilitate this procedure are at hand, including the phosphorylases. Since we are unable to identify the "smokeless" process, we cannot tell how many such procedures there really are, and though we know much about the hydrolytic glycolysis system, for which we suggested a "hook-

up" in the text, we do not know the connections whereby the energy carriers give up their energy to the functional mechanism. Several processes including the fluorescence we suggested may operate here. In view of the vast deficit of fact we must consider all explanations as provisional. Since fructose is oxidized so much easier than glucose we offer the above suggestion as a possible course consistent with established chemistry. (See Chapter II and III).

References to the Literature

References to supporting contributions well known to biologists are not listed with their publications. The time only of the publication is given. This is because it is better for anyone not informed on such developments to consult one of the many fine text books on biochemistry and obtain a workable view of the subject. Our own references are given because they did not follow the beaten paths and are not included in such reviews. Fact is always preferred to hypothesis, but where facts are inadequate a given goal has no other path than by hypothesis, and this was our predicament. However our hypotheses are founded on fact and established chemical theory, and we did reach our goal, as the clinical achievements demonstrate.

Alkalinization of the bowel to normal pH for patients under treatment.

Since we look upon the amines developed in the colon by bacterial decarboxylation of amino acids as the vanguard of disease in an important degree, we have to suggest a means of normalizing the reaction of the colon contents to a normal of pH 8 where decarboxylation does not take place. To accomplish this without injuring the acid secreting cells of the gastric mucosa, we *suggest the use of several mild alkalies* during the meals, *three times a day.*

Notice is taken of the fact that iron in the ferrous state inhibits some of the more important decarboxylases in only a one percent solution, while hydrazine inhibits the four "co-decarboxylase enzymes" practically completely in a dilution of but one to a thousand. The ferrous iron excites no contrary

mutation, while the hydrazine does. However the early improvement secured would prove helpful were it not that in cancer *this reagent must excite a more vigorous neoplasia.* No doubt in the recent tuberculosis therapy this inhibition of decarboxylation is a most important factor in the early improvement, but with adverse germ mutations that are inevitable, it is better to not use the reagent. A simple procedure of securing a normal intestinal alkalinity is all that is needed, so we have prepared a capsule which can be dissolved in water and taken during the course of the meal. Several alkalies are taken as the citrates and carbonates. These are, besides a small dose of iron, and minute traces of copper and cobalt, tin and zinc, material doses of sodium, potassium, calcium, magnesium, and manganese. The stools should be examined as to reaction, which should be held at pH 8 to pH 8.5. The amounts of alkali may then be increased or reduced as needed.

Prolonged Silent Symbiosis

After vaccination against small pox the virus may maintain a prolonged absorption into the tissues or an integration with cutaneous tissue material either as a living virus or as a toxin which is able to produce tissue changes of a pox nature generally over the surface of the body when this toxin or virus is subjected to the immunological changes consequent to the carbonyl therapy of our text. Thus at the twenty-eighth week after the treatment has been given to certain cases of cancer, and the neoplasms have about completed their absorption, a general eruption of "small-pox" or vaccinia may show up, and then clear in three weeks, as the terminal phase of the recovery process. We have not had enough experience to master the details of this reaction, but it appears to fall in line with the rule we proposed that during the recovery the first symptoms to show up are the last symptoms of the pathogenesis, and the last symptoms to appear are the first of the pathogenesis. Thus we would place *vaccination* or a mild small pox infection as a causative agent in the development of the neoplasm, or one of the causative agents. It is probably held all this time absorbed or copolymerized with the collagenous material of the protective fibrosis, and being first to be laid down in this tissue, it is

the last to be broken down in the structure of the protective fiber, and is the one to show the monomeric or original form of the toxin before polymerizing through the successive phases that produce differing symptoms as the pathogenesis goes on. The last change in the toxin when we meet it, is its neoplastic property, and hence in the reversal of structure back toward the monomeric original form, the carcinogenic form is first to be lost and the cause of the cancer is thus removed, so it undergoes dissolution first. Any intermediate symptoms of the pathogenesis would show up in reverse order to their coming and disappear before the monomeric pox forming structure is reached. Thus while this is being set free from the absorbing or copolymerizing fibrosis it may produce the original symptoms of the infection before it is fully burned out of the way. We hold that it is fully burned out of the way since there are no cicatricial sequelae to the affair. Where we have given the carbonyl treatment to deep scars as from burns and in keloids, or following pox, this fibrosis has also cleared up to a remarkable degree, so we conclude that the scar is what we say, a protective copolymere or structure of collagenous material that adds the free radicals or highly polar unoxidized double bonds of the toxin of germ origin, or some incompletely burned tissue cell metabolite, or of a heat product, or an integrated virus.

The toxic fibrosis may predominate in a given tissue as the vascular walls and nerve nodes to produce a malignant hypertension with a systolic pressure holding stubbornly at 280 mms. Hg. Then twelve weeks after treatment a few days of reaction may duplicate the symptoms of an old malaria of several decades previously. The blood pressure is then found to be 140/80m ms. Hg. and remains so while the other symptoms are forgotten. A "silent" malaria was a factor in the fibrosis. It was the cause of the "essential" hypertension.

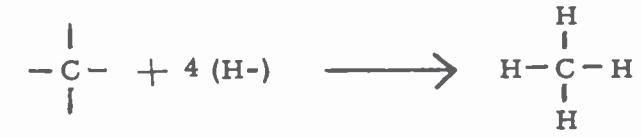
While vaccinia appears to form only the symbiotic type of integration, it may go lytic as would other less stabilized attenuated mutants, used as vaccines, when entering a favorable host. This may require hours or days. However certain viruses as Swine Pest virus are lytic only, and kill 100% of the pigs regularly in from 2 to 5 days. The state of union with the

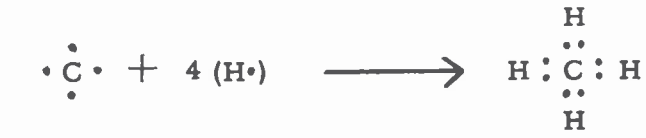
host cell however envolves the same atomic groups, as we have cured at times 100% of the treated pigs with one dose in from three to four days, even in the most virulent epidemics where the victims could not get up from the ground or eat or drink, and all untreated animals died in due course. The atomic unions are quite the same then no matter what form is taken or the state of the virulence. A twin or dimere status of the symbiotic virus holding unions as of Diagram V, (b), should be considered, as cleavage would favor change to the lytic type of action.

A Review of the Double Bond and the Free Radical

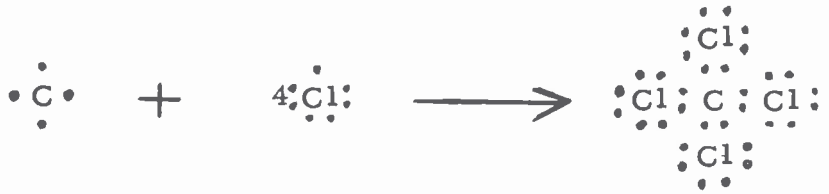
(1) Chemical Bonds:

The forces that hold atoms together to form molecules are called bonds. Each comprises a component or valence contributed by each atom. Up to 1859, when Kekule the renowned German chemist clarified the atom bonding system, progress was slow in establishing the structure of molecules. He proposed that the atom carbon holds four valences in all molecules, simple or complex, in which it takes part. Each valence he represented by a short line drawn out from the atom initial and joined to one from the other atom to represent the bond between them. This bond is termed the covalent bond for the reason just stated. It is made up of a pair of electrons shared by each atom. The following diagram illustrates (1) the Kekule formulae and (2) the electron formulae.

(1) 

(2) 

One will note that each atom donates and also accepts an electron to make up the bond. In such a molecule, all of the electrons and nuclear charges are in the same atomic field and hence the molecule is non-polar, non-ionized and stable in structure. Carbon atoms have relatively little tendancy to give up or accept electrons and hence tend not to form ionic bonds. Carbon does more readily combine with other atoms by electron sharing, which envolves much less energy expenditure than ionization, and hence is most favorable for biological structures and processes. It is also preferable to silicon for purposes of organic life. Carbon also tends to form a stable octet with electron accepting atoms such as chlorine, thus:



Carbon also gains stability through its four electrons in the outer shell with little tendancy to gain or contribute electrons, standing as it does midway between the stable electron states of Neon and Helium and being very different in type.

The Covalent Double Bond envolves the sharing of four electrons by two atoms, two electrons being contributed by each atom. The bond is shorter and the bond energy values are greater than for the covalent single bond. However, the mobile electrons are not entirely satisfied and tend to make additions. This may be surmised from the fact that the C-C bond energy is 58.6 kcal. while in the C=C double bond it is 100 kcal. per molecule, thus leaving about 17 kcal. looking for "satisfaction". In symetrical molecules the electrons are symetrically disposed. Otherwise one terminal of the double bond would present more than the other and be partially polarized. The substituents determine the direction in which electrons are asymmetrically displaced. Thus carbonyl and nitro substituents tend to polarize the electrons in the opposite direction, and like halogen tend

to decrease the availability of electrons in the bond. On the other hand, alkyl, aryl, alkoxy and ester groups increase the density of the electrons in the bond and tend to make it more negative. The tendency and position of addition of radicals carrying relatively positive or negative charges is thus determined. While methyl substituents tend to displace the mobile electrons in the opposite direction to make the closer pole of the double bond more positive and more able to attract a negatively charged atom or group, the situation may be reversed in the presence of peroxide catalysts. This is worth noting since peroxide catalysis is quite universal in tissue chemistry.

Some covalent double bonds are polarized by the charges the constituent atoms normally carry. Thus in the carbonyl group the carbon atom has an electro-negativity of 2.5 in comparison with oxygen's 3.5 and will tend to attract electrons from a conjugated ethylenic linkage, as well as a negatively charged atom, while the oxygen would tend to add the more positively charged unit. In the azomethine double bond the carbon electronegativity is less than that of the nitrogen, being 2.5 and 3.0 respectively, so the carbon would tend to attract the more negative oxygen first in the oxidative cleavage of the bond. A carbonyl group as a terminal is thus assured. The nitrogen would also attract oxygen and separate as an oxide of that nitrogen.

Double bonds tend also to mobilize the hydrogen atom attached to the carbon atom in the alpha position thereto. This hydrogen atom tends to separate as a proton. Continuous oxidative breakdown is thus facilitated with production of a peroxide free radical as a chain carrier, or of a terminal carbonyl group with each step of cleavage when an efficient dehydrogenator is supplied.

(2) Free Radicals:

The double bond may make an addition at one pole so as to break one bond with the other pole. This produces an unpaired electron or free radical at the other pole which wants to satisfy its stable octet by adding another electron. Whether this free radical is neutrophilic or electrophilic will depend on

the electronic concentration at that position. Free radicals so formed will add to one pole of a double bond of the same type molecule or another and thus form a dimer which presents a free radical at the opposite pole. These continue with the additions until the polymeres or copolymeres formed are so large in molecular weight and so inert that they fail to make further additions. Thus the polymerization process starts out with an initiator addition and continues as a propagation phase until it terminates itself by the inert nature of the final product. The propagation phase may be halted by addition of an inert free radical in like manner and at any stage of its progress.

In free radical production, the fission of a covalent bond is "homolytic". That is, each resultant fragment possesses an odd electron. One may contrast this situation with "heterolytic" fission in which the pair of electrons go with one fragment leaving the other with an exposed nucleus (ionization). The former fragment is negative and is nucleophilic (an anion), while the latter is positively charged and is electrophilic (a cation). Such rupture of a covalent bond results when the attacking atom has an affinity for an electron pair or for an atomic nucleus. A cation attacks at the point of highest electron availability while the anion where the greatest paucity of electrons is offered. The energy envolved in ionic reactions is much greater than where free radicals are produced, since two electrons are moved instead of one. So the latter are adapted to biochemical processes pre-eminently.

It is easy to see that when one dehydrogenator molecule can start a chain reaction that will increase in velocity with great speed and convert an enormous amount of substrate in short order, the concentration of the initiator need not be great. When acting on substances in solution, a minimum concentration as 1×10^{-12} will suffice. Such a solution still carries many millions of molecules where but one is required theoretically. When the reagent must initiate the reaction separately in a number of tissue cells, even though one molecule per tissue cell may suffice, the number of tissue cells must be considered and so the solution must be adequately concentrated or dilute as you please. Physiological reactions are of this order and that

speaks for their free radical and chain characters. Thus acetylcholine is found to renew contractions in surviving guinea pig intestine muscle in dilutions of one part to a thousand million of water. (Paul Karrer, Organic Chemistry, 3rd edition, 1947, p. 239). In this instance the hormone itself becomes the free radical. The quinones may offer free radical resonance hybrids which initiate reactions in equally high dilutions, and besides, the quinone carbonyl group may serve as a dehydrogenator and produce a free radical in the substrate from which the reaction is propagated. For example; Echinochrome A, the pentahydroxyl quinone of beta-ethylnaphthalene, produced in the ovary and egg of the Sea Urchin is able to motilize sperm cells in dilutions as high as one part to 2×10^9 parts of water. (Kuhn and Wallenfels 1939—Fieser & Fieser, Organic Chemistry, 1944 edition, pages 752-753). Likewise Crosin, whose dehydrogenator carbonyl group is activated by conjugation with an ethylene linkage, was shown by Kuhn and Moewus (1938-1940) to activate the gametes of certain algae in like high dilutions where but one molecule of the reagent served each sex cell. Benzoquinone has shown therapeutic effect in high dilution.

Diphenoquinone offers resonance hybrid free radicals in solution. When going into solution under correct conditions, an apple greenish yellow color is at first produced by diphenoquinone. This color lasts a few hours before turning to yellow and then becoming colorless as its polymeres separate out. The color may last as long as seven hours during which period it is therapeutically active, but after it becomes colorless it is inactive. The rich quota of resonance hybrids serve as chain initiating free radicals.

For this reason and because its highly activated carbonyl groups are such efficient dehydrogenators, its ability to initiate oxidation chains in high dilutions would be anticipated. Observations have shown that doses of a fraction of a microgram are sufficient to cure well established Hog Cholera in four days. To accomplish this, the amount of virus and toxin that had to be converted into peroxide free radical (Antitoxin State of Structure) to progagate the reaction was quite large. It was evident in the clinical symptoms that the rate of turnover in-

creased rapidly as the hours passed. In the treatment of Rabies, a small fraction of a microgram of a compound presenting serially arranged carbonyl groups with free radical terminals demonstrated the same conversion power. Molecular oxygen is necessary to the production of the "antitoxin" state of structure we referred to as early as 1924. It is the carrier of the oxidation chain so long as toxin is present, and thereafter persists as the "immune body" we referred to in "Cancer and its Allied Diseases" 1926. How this carrier is stabilized until new substrate arrives and how it is activated to again convert substrate is a matter of conjecture. Clinically and in animals we find it behaves that way, but the mechanisms are obscure still. A dimerization as when triphenylmethyl is converted to hexaphenylethane could be the case, or adsorption of the free radical into some colloidal structure, or perhaps the resonance hybrids discussed below. The subject is worth investigation.

The greenish yellow to pure yellow color of diphenoquinone, when under going solution in water, recalls the yellow color of triphenylmethyl which the writer observed while enjoying the courtesy of working in Prof. Gomberg's personal laboratory, just seven years after that wonderful gentleman and chemist demonstrated the free radical beyond any doubt. He found that his free radical gave the same absorption in the visible spectrum that quinoid structures show, as benzoquinone for example. This fact helped him work out the resonance hybrids, nine in number, that added to the stability of the structure. Because of this resonance triphenylmethyl as a free radical tends to show more stability than when dimerized to form hexaphenylethane. That is its resonance tends to weaken the C - C bond (70-80 kcal) which compensates for the energy needed to break the bond. The increase in entropy gained by the wandering of the lone electron of a free radical in this way should prove very instructive in considering the phenomena of the long persisting immunity. The lone electron in this capacity also determines the color characteristics and makes the molecule paramagnetic, both properties serving in their detection and measurements.

(3) Photoactivity:

Nitric oxide permanently presents a free radical stabilized as a covalent bond carrying three electrons. It can block chain reactions carried by free radicals. All oxides of nitrogen offer the same predicament; thus when nitrous oxide is used as an anaesthetic, it has sharply reversed many a splendid recovery course. This fact throws light on the nature of the recovery mechanism, indicating that it is a chain process carried by free radicals. Another experience is the blocking of the recovery course by substances carrying highly polarized double bonds. Substances in coffee, tea, in motor exhausts and paint solvents are of that nature. Substances that release hydrogen easily may reduce the free radical chain carrier and inactivate it so as to bring an end to the propagation of the reaction. If, however, the hydrogen donor is itself converted to a free radical and oxygen is at hand the oxidation chain may progress. Hindering substances are those with sulphidryl groups. The hydrogen is released to inactivate the carrier free radical and two dehydrogenated sulphur radicals will unite as a dimer, terminating the process. Free radicals act like cations preferring to attack positions of high electron density. Halogen anions and halogen free radicals thus act differently as the anion is nucleophilic (basophilic). This difference should be remembered in the interpretation of biochemical events and in the preparation of therapeutic agents.

Covalent bonds are split by light waves quite specifically. It takes more energy for this cleavage than when produced chemically or by heat since the atoms are activated by light in addition to the cleavage of the bond. The activation may be translational and result in more effective reactivity through increased collisions or it may be internal with transition of a valency electron from one quantum orbit to another, thus a new binding energy is obtained. In this excited state the molecule vibrates, and if the energy is sufficient, a dissociation into fragments may occur. When the energy is not sufficient, a rearrangement of atoms within the molecule may happen or a new electronic configuration may result. The reactivity is thus altered.

A molecule that has absorbed radiant energy is capable of fluorescence, that is the emission of this energy (somewhat degraded) as radiation almost immediately (10^{-8} sec.) after its reception, unless the energy has been used in some other way as by collision with a particle that can use the exact energy quantum that the activated molecule is carrying. This specificity is important to our photosensitization theory of carcinogenesis (Koch, Natural Immunity, 1934, 1936, 1939). The gas phase is ideal for emission as irradiation, as the closer the molecules are together and the denser the medium, the greater the opportunity for inactivating collisions. Such inactivation by collision is a very specific affair and only molecules of a particular molecular species can accept the energy available and become inactivated thereby. Particles that can quench fluorescence in this way gain the energy as internal energy and not as translational energy. This type of activation is termed photosensitization. Such energy may bring about dissociation of covalent bonds and produce free radicals that bring about activation of reactions that mediate the cycle of nucleoprotein construction and cleavage of viral and neoplastic propagation. Once the free radical is formed, whether by light energy or in some chemical way discussed earlier, its deportment follows the known behaviors of free radicals. The initiation of carcinogenesis need not be by irradiation, though the processes of photosensitization depending on the energy passed internally through collisions can produce free radicals and the ultimate effect is the same as if from irradiation.