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Gentlemen:

The attached reprint recently came to my attention and I thought each of you might be interested in reading it since it discusses some of the fundamentals of carcinogenesis.

It is in my mind a most entertaining theory on the origin of cancer cells, although I understand it is not universally accepted by those in the cancer research field. However, it is a well written article and I think would be of interest to each of you because of your activities with our Subcommittee.

Very truly yours,

[illegible], M. D.

[illegible]
[illegible] - On the Origin of Cancer Cells, O. Warburg. SCIENCE, 123:309, 2/24/56.

cc: Mr. [illegible] - w/att.

API 06161

SCIENCE

On the Origin of Cancer Cells

Otto Warburg

Our principal experimental object for the measurement of the metabolism of cancer cells is today no longer the tumor but the ascites cancer cells (1) living free in the abdominal cavity, which are almost pure cultures of cancer cells with which one can work quantitatively as in chemical analysis. Formerly, it could be said of tumors, with their varying cancer cell content, that they ferment more strongly the more cancer cells they contain, but today we can determine the absolute fermentation values of the cancer cells and find such high values that we come very close to the fermentation values of wildly proliferating *Torula* yeasts.

What was formerly only qualitative has now become quantitative. What was formerly only probable has now become certain. The era in which the fermentation of the cancer cells or its importance could be disputed is over, and no one today can doubt that we understand the origin of cancer cells if we know how their large fermentation originates, or, to express it more fully, if we know how the damaged respiration and the excessive fermentation of the cancer cells originate.

Energy of Respiration and Fermentation

Today we understand the chemical mechanisms of respiration and fermentation [illegible], but we do not need [illegible] for what follows [illegible] will be the center of [illegible]. We need to know [illegible] respiration and fermentation [illegible] are energy-producing reactions and that they synthesize the energy-rich adenosine triphosphate, through which the energy of respiration and fermentation is then made available for life. Since it is known how much adenosine triphosphate can be synthesized by respiration and how much by fermentation, we can write immediately the potential, biologically utilizable energy production of any cells if we have measured their respiration and fermentation. With the ascites cancer cells of the mouse, for example, we find an average respiration of 7 cubic millimeters of oxygen consumed per milligram, per hour, and fermentation of 60 cubic millimeters of lactic acid produced per milligram, per hour. This, converted to energy equivalents, means that the cancer cells can obtain approximately the same amount of energy from fermentation as from respiration, whereas the normal body cells obtain much more energy from respiration than from fermentation. For example, the liver and kidney of an adult animal obtain about 100 times as much energy from respiration as from fermentation.

I shall not consider aerobic fermentation, which is a result of the interaction of respiration and fermentation, because aerobic [illegible] too dependent on external conditions. Of importance [illegible] considerations that follow [illegible] respiration and [illegible] respiration, which is measured by the oxygen consumption of [illegible] saturated with oxygen, and [illegible] measured [illegible]

Injuring of Respiration

Since the respiration of all cancer cells is damaged, our first question is, How can the respiration of body cells be injured? Of this damage to respiration, it can be said at the outset that it must be irreversible, since the respiration of cancer cells never returns to normal. Second, the injury to respiration must not be so great that the cells are killed, for then no cancer could result. If respiration is damaged when it forms too little adenosine triphosphate, it may be either that the oxygen consumption has been decreased or that, with undiminished oxygen consumption, the coupling between respiration and the formation of adenosine triphosphate has been broken, as was first pointed out by Feodor Lynen (2).

One method for the destruction of the respiration of body cells is removal of oxygen. If, for example, embryonal tissue is exposed to an oxygen deficiency for some hours and then is placed in oxygen again, 50 percent or more of the respiration is usually destroyed. The cause of this destruction of respiration is lack of energy. As a matter of fact, the cells need their respiratory energy to preserve their structure, and if respiration is inhibited, both structure and respiration disappear.

Another method for destroying respiration is to use respiratory poisons. From the standpoint of energy, this method comes to the same result as the first method. No matter whether oxygen is withdrawn from the cell or whether the oxygen is prevented from reacting by a poison, the result is the same in both cases, namely, impairment of respiration from lack of energy.

I may mention a few respiratory poisons. A strong, specific respiratory poison is arsenious acid, which, as every [illegible], may produce cancer. Hydrogen sulfide and many of its deriv-

The author, Otto Warburg, is director of the Max Planck Institute for Cell Physiology, Berlin-Dahlem, Germany. This article is based on a lecture [illegible] at Stuttgart on 25 May 1955 before the German Central Committee for Cancer Control. It was [illegible] in German in [illegible] 42, 401 (1955). This translation was prepared by Dean Burk, Jehu Hunter, [illegible] of the U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, Md., with the permission of [illegible] and with the collaboration of Professor Warburg [illegible]

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atives are also strong, specific respiratory poisons. We know today that certain hydrogen sulfide derivatives, thiourea and thioacetamide, with which citrus fruit juices have been preserved in recent times, induce cancer of the liver and gall bladder in rats.

Urethane is a nonspecific respiratory poison. It inhibits respiration as a chemically indifferent narcotic, since it displaces metabolites from cell structures. In recent years it has been recognized that subnarcotic doses of urethane cause lung cancer in mice in 100 percent of treatments. Urethane is particularly suitable as a carcinogen, because, in contrast to alcohol, it is not itself burned up on the respiring surfaces and, unlike ether or chloroform, it does not cytolyze the cells. Any narcotic that has these properties may cause cancer upon chronic administration in small doses.

The first notable experimental induction of cancer by oxygen deficiency was described by Goldblatt and Cameron (*3*), who exposed heart fibroblasts in tissue culture to intermittent oxygen deficiency for long periods and finally obtained transplantable cancer cells, whereas in the control cultures that were maintained without oxygen deficiency, no cancer cells resulted. Clinical experiences along these lines are innumerable: the production of cancer by intermittent irritation of the outer skin and of the mucosa of internal organs, by the plugging of excretory ducts of glands, by cirrhoses of tissues, and so forth. In all these cases, the intermittent irritations lead to intermittent circulatory disturbances. Probably chronic intermittent oxygen deficiency plays a greater role in the formation of cancer in the body than does the chronic administration of respiratory poisons.

Any respiratory injury due to lack of energy, however, whether it is produced by oxygen deficiency or by respiratory poisons, must be cumulative, since it is irreversible. Frequent small doses of respiratory poisons are therefore more dangerous than a single large dose, where there is always the chance that the cells will be killed rather than that they will become carcinogenic.

Grana

If an injury of respiration is to produce cancer, this injury must, as already mentioned, be irreversible. We understand by this not only that the inhibition of respiration remains after removal of the respiratory poison but, even more, that the inhibition of respiration also continues through all the following cell divisions, for measurements of metabolism in transplanted tumors have shown that cancer cells cannot regain normal respiration, even in the course of many decades, once they have lost it.

This originally mysterious phenomenon has been explained by a discovery that comes from the early years of cell physiology (*4*). When liver cells were cytolyzed by infusion of water and the cytolyzate was centrifuged, it was found that the greater part of the respiration sank to the bottom with the cell grana. It was also shown that the respiration of the centrifuged grana was inhibited by narcotics at concentrations affecting cell structures, from which it was concluded —already in 1914—that the respiring grana are not insoluble cell particles but *autonomous organisms*, a result that has been extended in recent years by the English botanist Darlington (*5*) and particularly by Mark Woods and H. G. du Buy (*6*) of the National Cancer Institute in Bethesda, Md. Woods and du Buy have experimentally expanded our concepts concerning the self-perpetuating nature of mitochondrial elements (grana) and have demonstrated the hereditary role of extranuclear aberrant forms of these in the causation of neoplasia. The autonomy of the respiring grana, both biochemically and genetically, can hardly be doubted today.

If the principle *Omne granum e grano* is valid for the respiring grana, we understand why the respiration connected with the grana remains damaged when it has once been damaged; it is for the same reason that properties linked with genes remain damaged when the genes have been damaged.

Furthermore, the connection of respiration with the grana (*7*) also explains a carcinogenesis that I have not mentioned previously, the carcinogenesis by x-rays. Rajewsky and Pauly have recently shown that the respiration linked with the grana can be destroyed with strong doses of x-rays, while the small part of the respiration that takes place in the fluid protoplasm can be inhibited very little by irradiation. Carcinogenesis by x-rays is obviously nothing else than a destruction of respiration by elimination of the respiring grana.

It should also be mentioned here that grana, as Graffi has shown (*8*), fluoresce brightly if carcinogenic hydrocarbons are brought into their surroundings, because the grana accumulate the carcinogenic substances. Probably this accumulation is the explanation for the fact that carcinogenic hydrocarbons, although almost insoluble in water, can inhibit respiration and therefore have a carcinogenic effect.

Increase of Fermentation

When the respiration of body cells has been irreversibly damaged, cancer cells by no means immediately result. For cancer formation there is necessary not only an irreversible damaging of the respiration but also an increase in the fermentation—indeed, such an increase of the fermentation that the failure of respiration is compensated for energetically. But how does this increase of fermentation come about?

The most important fact in this field is that there is no physical or chemical agent with which the fermentation of cells in the body can be increased directly; for increasing fermentation, a long time and many cell divisions are always necessary. The temporal course of this increase of fermentation in carcinogenesis has been measured in many interesting works, among which I should like to make special mention of those of Dean Burk (*9*).

Burk first cut out part of the liver of healthy rats and investigated the metabolism of the liver cells in the course of the ensuing regeneration, in which, as is well known, the liver grows more rapidly than a rapidly growing tumor. No increase of fermentation was found. Burk then fed rats for 200 days on butter yellow, whereupon liver carcinomas were produced, and he found that the fermentation slowly increased in the course of 200 days toward values characteristic of tumors.

The mysterious latency period of the production of cancer is, therefore, nothing more than the time in which the fermentation increases after a damaging of the respiration. This time differs in various animals; it is especially long in man and here often amounts to several decades, as can be determined in the cases in which the time of the respiratory damage is known—for example, in arsenic cancer and irradiation cancer.

The driving force of the increase of fermentation, however, is the energy deficiency under which the cells operate after destruction of their respiration, which forces the cells to replace the irretrievably lost respiration energy in some way. They are able to do this by a selective process that makes use of the fermentation of the normal body cells. The more weakly fermenting body cells perish, but the more strongly fermenting ones remain alive, and this selective process continues until the respiratory failure is compensated for energetically by the increase in fermentation. Only then has a cancer cell resulted from the normal body cell.

Now we understand why the increase in fermentation takes such a long time and why it is possible only with the help of many cell divisions. We also understand why the latency period is different in rats and in man. Since the average fermentation of normal rat cells is much greater than the average fermentation

of normal human cells, the selective process begins at a higher fermentation level in the rat and, hence, is completed more quickly than it is in man.

It follows from this that there would be no cancers if there were no fermentation of normal body cells, and hence we should like to know, naturally, from where the fermentation of the normal body cells stems and what its significance is in the body. Since, as Burk has shown, the fermentation remains almost zero in the regenerating liver growth, we must conclude that the fermentation of the body cells has nothing to do with normal growth. On the other hand, we have found that the fermentation of the body cells is greatest in the very earliest stages of embryonal development and that it then decreases gradually in the course of embryonal development. Under these conditions, it is obvious—since ontogeny is the repetition of phylogeny—that the fermentation of body cells is the inheritance of undifferentiated ancestors that have lived in the past at the expense of fermentation energy.

Structure and Energy

But why—and this is our last question—are the body cells dedifferentiated when their respiration energy is replaced by fermentation energy? At first, one would think that it is immaterial to the cells whether they obtain their energy from respiration or from fermentation, since the energy of both reactions is transformed into the energy of adenosine triphosphate, and yet adenosine triphosphate = adenosine triphosphate. This equation is certainly correct chemically and energetically, but it is incorrect morphologically, because, although respiration takes place for the most part in the structure of the grana, the fermentation enzymes are found for a greater part in the fluid protoplasm. The adenosine triphosphate synthesized by respiration therefore involves more structure than the adenosine triphosphate synthesized by fermentation. Thus, it is as if one reduced the same amount of silver on a photographic plate by the same amount of light, but in one case with diffused light and in the other with patterned light. In the first case, a diffuse blackening appears on the plate, but in the second case, a picture appears; however, the same thing happens chemically and energetically in both cases. Just as the one type of light energy involves more structure than the other type, the adenosine triphosphate energy involves more structure when it is formed by respiration than it does when it is formed by fermentation.

In any event, it is one of the fundamental facts of present-day biochemistry that adenosine triphosphate can be synthesized in homogeneous solutions with crystallized fermentation enzymes, whereas so far no one has succeeded in synthesizing adenosine triphosphate in homogeneous solutions with dissolved respiratory enzymes, and the structure always goes with oxidative phosphorylation.

Moreover, it was known for a long time before the advent of crystallized fermentation enzymes and oxidative phosphorylation that fermentation—the energy-supplying reaction of the lower organisms—is morphologically inferior to respiration. Not even yeast, which is one of the lowest forms of life, can maintain its structure permanently by fermentation alone; it degenerates to bizarre forms. However, as Pasteur showed, it is rejuvenated in a wonderful manner if it comes in contact with oxygen for a short time. "I should not be surprised," Pasteur said in 1876 (*10*) in the description of these experiments, "if there should arise in the mind of an attentive hearer a presentiment about the causes of those great mysteries of life which we conceal under the words youth and age of cells." Today, after 80 years, the explanation is as follows: the firmer connection of respiration with structure and the looser connection of fermentation with structure.

This, therefore, is the physicochemical explanation of the dedifferentiation of cancer cells. If the structure of yeast cannot be maintained by fermentation alone, one need not wonder that highly differentiated body cells lose their differentiation upon continuous replacement of their respiration with fermentation.

I would like at this point to draw attention to a consequence of practical importance. When one irradiates a tissue that contains cancer cells as well as normal cells, the respiration of the cancer cells, already too small, will decline further. If the respiration falls below a certain minimum that the cells need unconditionally, despite their increased fermentation, they die; whereas the normal cells, where respiration may be harmed by the same amount, will survive because, with a greater initial respiration, they will still possess a higher residual respiration after irradiation. This explains the selective killing action of x-rays on cancer cells. But still further: the descendants of the surviving normal cells may in the course of the latent period compensate the respiration decrease by fermentation increase and, thence, become cancer cells. Thus it happens that radiation which kills cancer cells can also at the same time produce cancer or that urethane, which kills cancer cells, can also at the same time produce cancer. Both events take place from harming respiration, the killing, by harming an *already* harmed respiration, the carcinogenesis by the harming of a *not yet* harmed respiration.

Maintenance Energy

When dedifferentiation of the body cells has occurred and cancer cells have thereby developed, there appears a phenomenon to which our attention has been called by the special living conditions of the ascites cancer cells. In extensively progressed ascites cancer of the mouse, the abdominal cavity contains so many cancer cells that the latter cannot utilize their full capacity to respire and ferment because of the lack of oxygen and sugar. Nevertheless, the cancer cells remain alive in the abdominal cavity, as the result of transplantation proves.

Recently we have confirmed this result by direct experiments in which we placed varying amounts of energy at the disposal of the ascites outside the body, *in vitro*, and then transplanted it. This investigation showed that all cancer cells were killed when no energy at all was supplied for 24 hours at 38°C but that one-fifth of the growth energy was sufficient to preserve the transplantability of the ascites. This result can also be expressed by saying that cancer cells require much less energy to keep them alive than they do for growth. In this they resemble other lower cells, such as yeast cells, which remain alive for a long time in densely packed packets—almost without respiration and fermentation.

In any case, the ability of cancer cells to survive with little energy, if they are not growing, will be of great importance for the behavior of the cancer cells in the body.

Sleeping Cancer Cells

Since the increase in fermentation in the development of cancer cells takes place gradually, there must be a transitional phase between normal body cells and fully formed cancer cells. Thus, for example, when fermentation has become so great that dedifferentiation has commenced, but not so great that the respiratory defect has been fully compensated for energetically by fermentation, we may have cells which indeed look like cancer cells but are still energetically insufficient. Such cells, which are clinically not cancer cells, have lately been found, not only in the prostate, but also in the lungs, kidney, and stomach of elderly persons. Such cells have been referred to as "sleeping cancer cells" (*11, 12*).

The sleeping cancer cells will possibly play a role in chemotherapy. From energy considerations, I could think that

[illegible] had more [illegible] in the [illegible] test objects [illegible] agents [illegible] cancer cells of skin—that is, precancerous skin.

Summary

Cancer cells originate from normal body cells in *two* phases. The first phase is the irreversible injuring of respiration. Just as there are many remote causes of plague—heat, insects, rats—but only one common cause, the plague bacillus, there are a great many remote causes of cancer—tar, rays, arsenic, pressure, urethane—but there is only one common cause into which all other causes of cancer merge, the irreversible injuring of respiration.

The irreversible injuring of respiration is followed, as the second phase of cancer formation, by a long struggle for existence by the injured cells to maintain their structure, in which a part of the cells perish from lack of energy, while another part succeed in replacing the irretrievably lost respiration energy by fermentation energy. Because of the morphological inferiority of fermentation energy, the highly differentiated body cells are converted by this into undifferentiated cells that grow wildly—the cancer cells.

To the thousands of quantitative experiments on which these results are based, I should like to add, as a further argument, the fact that there is no alternative today. If the explanation of a vital process is its reduction to physics and chemistry, there is today no other explanation for the origin of cancer cells, either special or general. From this point of view *mutation* and *carcinogenic agent* are not alternatives, but empty words, unless metabolically specified. Even more harmful in the struggle against cancer can be the continual discovery of miscellaneous cancer agents and cancer viruses, which, by obscuring the underlying phenomena, may hinder necessary preventive measures and thereby become responsible for cancer cases.

Technical Considerations and Comments

Metabolism of the ascites cancer cells. The high fermentation of ascites cancer cells was discovered in Dahlem in 1951 (*12*) and since then has been confirmed in many works (*13, 14*). For best measurements, the ascites cells are not transferred to Ringer's solution but are maintained in their natural medium, ascites serum, which is adjusted physiologically at the beginning of the measu[illegible] [illegible] [illegible] and [illegible]. Because [illegible] large fermentation it is necessary to dilute the [illegible] cells that are removed from the abdominal cavity rather considerably with ascites serum, otherwise the bicarbonate would be used up within a few minutes after addition of the glucose, and hence the fermentation would be brought to a standstill.

Under physiological conditions of pH and temperature, we find the following metabolic quotients in ascites serum (*15*):

$$Q_{O_2} = -[illegible] \text{ to } [illegible]$$

$$Q_M^{O_2} = 25 \text{ to } 35$$

$$Q_M^{N_2} = 50 \text{ to } 70$$

where Q_{O_2} is the amount of oxygen in cubic millimeters that 1 milligram of tissue (dry weight) consumes per hour at 38°C with oxygen saturation, $Q_M^{O_2}$ is the amount of lactic acid in cubic millimeters that 1 milligram of tissue (dry weight) develops per hour at 38°C with oxygen saturation, and $Q_M^{N_2}$ is the amount of lactic acid in cubic millimeters that 1 milligram of tissue (dry weight) develops per hour at 38°C in the absence of oxygen.

Even higher fermentation quotients have been found in the United States with other strains of mouse ascites cancer cells (*13, 14*).

All calculations of the energy-production potential of cancer cells should now be based on the quotients of the ascites cancer cells, since these quotients are 2 or 3 times as large anaerobically as the values formerly found for the purest solid tumors. The quotients of the normal body cells, however, remain as they were found in Dahlem in the years from 1924 to 1929 (*16–19*). It is clear that the difference in metabolism between normal cells and cancer cells is much greater than it formerly appeared to be on the basis of measurements on solid tumors.

Utilizable energy of respiration and fermentation. Since the discovery of the oxidation reaction of fermentation in 1939 (*20*), we have known the chemical reactions by which adenosine diphosphate is phosphorylated to adenosine triphosphate in fermentation, and since then we have found that 1 mole of fermentation lactic acid produces 1 mole of adenosine triphosphate (ATP).

The chemical reactions by which ATP is synthesized in respiration are still unknown but it can be assumed, according to the existing measurements (*21*), that 7 moles of ATP can be formed when 1 mole of oxygen is consumed in respiration.

ATP quotients. If we multiply Q_{O_2} by [illegible] and $Q_M^{N_2}$ by 1, we obtain the number of [illegible] millimeters of ATP that [illegible] milligram of tissue (dry substance) [illegible] [illegible] per hour (22,400 cubic m[illegible] [illegible] mole [illegible] ATP). We call these quotients $Q_{ATP}^{O_2}$ and $Q_{ATP}^{N_2}$, according to whether the ATP is formed by respiration or by fermentation, respectively.

Energy production of cancer cells and of normal body cells. In Table [illegible] the Q values of some normal body cells are contrasted with the Q values of our ascites cancer cells.

The cancer cells have about as much energy available as the normal body cells, but the ratio of the fermentation energy to the respiration energy is much greater in the cancer cells than it is in the normal cells.

Uncoupling of respiration. If [illegible] [illegible] embryo is transferred from the amniotic [illegible] to Ringer's solution, the previously transparent embryo becomes opaque and soon appears [illegible] (*17*). At the same time, the [illegible] between respiration and phosphorylation is broken; that is, although oxygen is still consumed and carbon dioxide is still developed, the energy of th[illegible] [illegible] process is lost for life. If the metabolism quotients had previously been

$$Q_{O_2} = -15, \; Q_M^{O_2} = 0, \; Q_M^{N_2} = 25$$

$$Q_{ATP}^{O_2} = 105, \; Q_{ATP}^{N_2} = 2[illegible]$$

in the amniotic fluid, afterward, in Ringer's solution, they are

$$Q_{O_2} = -15, \; Q_M^{O_2} = 25, \; Q_M^{N_2} = [illegible]5$$

$$Q_{ATP}^{O_2} = 0, \; Q_{ATP}^{N_2} = 25$$

Because of uncoupling of respiration and phosphorylation, the energy production of the embryo has fallen from $Q_{ATP}^{O_2} + Q_{ATP}^{N_2} = 130$, to 25; since the uncoupling is irreversible, the embryo dies in the Ringer's solution.

This example will show that the first phase of carcinogenesis, the irreversible damaging of respiration, need not be an actual decrease in the respiration quotient but merely an uncoupling of respiration, with undiminished over-all oxygen consumption. Ascites cancer cells, which owe their origin primarily to an uncoupling of respiration, could conceivably have the following metabolism quotients, for example:

$$Q_{O_2} = -50, \; Q_M^{O_2} = 100, \; Q_M^{N_2} = [illegible]$$

$$Q_{ATP}^{O_2} = 0, \; Q_{ATP}^{N_2} = [illegible]$$

which would mean that, despite great respiration, the usable energy production would be displaced completely [illegible] [illegible] side of fermentation. O[illegible] [illegible] [illegible] have to search for such [illegible] [illegible] among the ascites cancer [illegible]. Solid [illegible]mors—and especially solid [illegible] [illegible] tumors—need no longer [illegible] subjected [illegible] such examinations [illegible] of course since the solid tumors are [illegible] [illegible] [illegible] histologica.

[illegible] fermentation [illegible] [illegible] fermentation [illegible] a property [illegible] growing cancer cells but aerobic fermentation

without [illegible] body [illegible] have [illegible] from amniotic fluid [illegible] R[illegible] Since [illegible] [illegible] but generally difficult to detect growth. [illegible] body cells, aerobic [illegible] be used as a test [illegible] made clear in 1928 [illegible]

[illegible] is still made of aerobic [illegible] Thus O'Connor [illegible] recently repeated our [illegible] experiments on the aerobic fermentation of the [illegible] that has been transferred into Ringer's solution, but he drew the conclusion that the growth of normal body cells is completed at the expense of the [illegible] fermentation, even though it has long been established that the embryo does not ferment aerobically when it grows in the amniotic fluid.

Respiratory poisons. The specific respiration-inhibiting effect of arsenious acid and the irreversibility of its inhibitions were discovered in the first quantitative works on cell respiration (23, 24). There is abundant literature on the carcinogenesis by arsenic, particularly on arsenic cancer after treatment of psoriasis and on the cancer of grape owners who spray their vineyards with arsenic. The specific respiration-inhibiting effect of hydrogen sulfide has likewise been described by Negelein (25), and carcinogenesis by derivatives of hydrogen sulfide has been recently described by D. N. Gupta (26).

The irreversible inhibition of cell respiration by urethane was discovered early (27) as well as the fact that the urethane inhibition is more irreversible the higher the temperature. In sea urchin eggs, the effect of urethane was investigated, not only on the metabolism, but also on cell division, in studies (28) from which the later urethane treatment of leukemia was developed. The physicochemical mechanism by which urethane and other indifferent narcotics inhibit cell respiration was cleared up in 1921 (29). Only much later did the carcinogenic effect of urethane become known. Actually, multiple lung adenomas can often be produced in 100 percent of the mice treated with small doses of urethane (30).

Oxygen deficiency. Short-period oxygen deficiency irreversibly destroys the respiration of embryos (16) without thereby inhibiting the anaerobic fermentation [illegible] are [illegible] (31). It has [illegible] that in the development of the [illegible] salamander, malformations [illegible] the respiration was [illegible] but [illegible] acid in the early [illegible] embryonal development (32).

Goldblatt and Cameron (33) reported that, in the [illegible] culturing of fibroblasts, tumor cells appeared when the cultures were exposed to intermittent oxygen deficiency for long periods, whereas in the control cultures no tumor cells appeared. In the discussion at the Stuttgart convention, Letté cited against Goldblatt and Cameron the fact that another American tissue culturist, Earle, has occasionally obtained tumor cells from fibroblasts for reasons unknown to him and in an unreproduceable manner, but this objection does not seem weighty, and the latter part is untrue (33). In any event, here is an area in which the methods of tissue culture could prove useful for cancer research. But warnings must be given against metabolism measurements in tissue cultures, if and when the tissue cultures are mixtures of growing and dying cells, especially under conditions of malnutrition. An example of the latter type of confusion is involved in the discussion by Albert Fischer (34), especially in the chapter "Energy exchange of tissue cells cultivated *in vitro*."

Rous agent. If the Rous agent is inoculated into the chorion of chick embryos, tumors originate in the course of a few days—as rapidly as in the transplantation of cancer cells. The tumors formed are not chorion tumors but Rous sarcomas. The Rous agent, to which a particle weight of 150 million is ascribed at present, is therefore capable of transmitting the morphological properties of the Rous sarcoma; and whatever we call the Rous agent—"hereditary unit," cell fragment, microcell, or spore—the transmission of the Rous sarcoma by the Rous agent is, in any case, nothing more than a *transplantation* and is to be differentiated strictly from the production of a chicken sarcoma by methylcholanthrene, which is a *neoformation* of a tumor from normal body cells and as such takes a long time.

The metabolism of the chicken sarcomas, whether produced by the Rous agent or by methylcholanthrene, is the [illegible] (35). In the first case, however, the fermentation potential has been transplanted with the Rous agent, whereas in the second case the fermentation has been intensified by selection from normal body cells under the action of methylcholanthrene.

Addendum: in vitro Carcinogenesis and Metabolism

Since this paper was prepared, striking confirmation and extension of its main conclusions have been obtained from correlated metabolic and growth studies of two lines of tissue culture cancer cells of widely differing malignancy that were both derived from *one and the same* normal, tissue-culture cell (36). The single cell was isolated some 5 years ago from a 97-day old parent culture of normal subcutaneous adipose tissue of a strain C3H/He mouse by Sanford, Likely, and Earle (37) of the National Cancer Institute. Up to the time that the single-cell isolation was made, no tumors developed when cells of the parent culture were injected into strain C3H/He mice. Injections of *in vitro* cells of the lines 1742 and 2049 (formerly labeled substrains VII and III, respectively) first produced tumors in normal C3H/He mice after the 12th and 19th *in vitro* transplant generations, respectively. After 1½ years, the percentage production of sarcomas was 63 and 0 percent, respectively; and after 3 years, it was 97 and 1 percent, respectively, with correspondingly marked differences in length of induction period. Despite such gross differences in "malignancy" *in vivo*, the rates of growth of the two lines of cells maintained continuously *in vitro* have remained nearly identical and relatively rapid. Nevertheless, the metabolism of the two lines of cancer cells, whose malignancy was developed *in vitro*, has been found by Woods, Hunter, Hobby, and Burk to parallel strikingly the differences in malignancy observed *in vivo*, in a manner in harmony with the predications and predictions of this article.

The metabolic values were measured following direct transfer of the liquid cultures from the growth flasks into manometric vessels, without notable alteration of environmental temperature, pH, or medium composition (horse serum, chick embryo extract, glucose, bicarbonate, balanced saline). The values obtained thus accurately represent the metabolism of growing, adequately nourished, pure lines of healthy cancer cells free of admixture with any other tissue cell type. The anaerobic glycolysis of the high-malignancy line 1742 was $Q_M^{N_2}$ = 60 to 80, which is virtually max-

Table 1. Contrast of the Q values of some normal body cells with the Q values of ascites cancer cells

Cells	Q_{O_2}	$Q_M^{N_2}$	Q^{O_2}	Q^{N_2}	$Q^{O_2} + Q^{N_2}$
Liver	−15	1	105	1	106
Kidney	−15	1	105	1	106
Embryo (very young)	−15	25	105	25	130
Cancer	−7	60	49	60	109

[illegible] cells previously [illegible] using ascites cells [illegible]. The anaerobic glycolysis of the low-malignancy line was, however, only one-third as great, $Q_M^{N_2}$ = 20 to 30. The average aerobic glycolysis values for the two lines were in the same order, $Q_M^{O_2}$ = 30 and 10, respectively, but of lower magnitude because of the usual, pronounced Pasteur effect, greater in line 1742 than in line 2049 ($Q_M^{N_2} - Q_M^{O_2}$ = about 80 and [illegible]). On the other hand, the rates of oxygen consumption were in the converse order, being smaller in line 1742 (Q_{O_2} = 5 to 10) than in line 2049 (Q_{O_2} = 10 to 15), corresponding to a greater degree of respiratory defect in line 1742. The respiratory defect in both lines was further delineated by the finding of little or no increase in respiration after the addition of succinate to either line of cells, in contrast to the considerable increases obtained with virtually all normal tissues (9), and the respiratory increase with paraphenylenediamine was likewise relatively low, compared with normal tissue responses.

A further notable difference between the two cell lines was the very much lower inhibition of glycolysis by podophyllin materials (anti-insulin potentiators) observed with line 1742 compared with line 2049 (for example, 10 and 70 percent, respectively, at a suitably low concentration). This result would be expected on the basis of the much greater loss of anti-insulin hormonal restraint of glucose metabolism, at the hexokinase phosphorylating level, as the degree of malignancy is increased, just as was reported for a spectrum of solid tumors (14).

Finally, the high-malignancy line 1742 cells have been found by A. L. Schade to contain 3 times as much aldolase as the low-malignancy line 2049 cells (11,- 300 versus 3700 Warburg activity units per milliliter of packed cells extracted), and about 2 times as much α-glycerophosphate dehydrogenase (2600 versus [illegible] Schade activity units (13) per milliliter of packed cells extracted). The potential significance of these indicated enzymic differences in relation to the parallel glycolytic differences, measured with aliquots of the same cell cultures, is evident, and may well be connected with the corresponding hexokinase system differences.

The new metabolic data on the two remarkably contrasting lines of cancer cells, which originated from a single, individual cell and have been maintained exclusively *in vitro* over a period of years, epitomize and prove finally the main conclusions of this article, which are based on decades of research. Such metabolic analyses provide promise of a powerful tool for diagnosis of malignancy in the ever-increasing variety of tissue-culture lines now becoming available in this rapidly expanding biological and medical field, where characterization of malignancy by conventional methods (animal inoculation or otherwise) may be difficult or impracticable. This metabolic tool should be especially important in connection with the use of tissue cultures for the evaluation of chemotherapeutic agents or other control procedures.

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36. This summary of studies [illegible] ing groups of investigators [illegible] ated by Dean Burk at Professor W[illegible] [illegible]

A university has its responsibility to the student and [illegible] an obligation to add to knowledge. Let us not put our students in the position of [illegible] or being a party to practices contrary to the ideal for a university and for his future [illegible]. If the student [illegible] institution engaged in fundamental research, [illegible] free, [illegible] free, [illegible] learn that independence in investigation is man's right [illegible] a university [illegible] exemplify.—N. Paul Hudson, Ohio State University [illegible] School [illegible] December 1953.

API 06167

carcinogenicity

ESSO RESEARCH AND ENGINEERING COMPANY

(FORMERLY STANDARD OIL DEVELOPMENT COMPANY)

P. O. Box 121, Linden, N. J.

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CHIEF CLINICAL RESEARCH PHYSICIAN

July 6, 1956

Mr. David V. Stroop
American Petroleum Institute
50 West 50th Street
New York 20, New York

Dear Mr. Stroop:

The attached information has been received from the Medical Research Council in England, describing their work on the carcinogenic action of mineral oils.

You will note that in attachment "B" mention is made of a series of sheets which have been compiled by them which I have removed from this information and will keep in the Subcommittee on Carcinogenicity file as a permanent record. I do not believe these tables would be of any particular interest to the Medical Advisory Committee but I do believe that they would be interested in the enclosed information.

I would appreciate it if you would have this information duplicated and distributed to the members of the Medical Advisory Committee.

Very truly yours,

R. E. Eckardt

R. E. ECKARDT, M. D.

REE:cjh
Attach. - Minutes of the Committee on the
Carcinogenic Action of Mineral Oils
20th Meeting, July 3, 1956

Copy From D. V. Stroop For Information Of Members of Medical Advisory Committee 7/9/56

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MEDICAL RESEARCH COUNCIL

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MRC.56/608
CAMO.Ag.20

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

A G E N D A

for the Twentieth Meeting to be held at
38 Old Queen Street, Westminster, S. W. 1,
on Tuesday, 3rd July, 1956, at 2.15 p. m.

1. Minutes of the previous meeting (MRC.55/1010, CAMO. Min.19 - already circulated).

2. Progress Reports:-

   (i) Chemical -

      (a) by Dr. W. Carruthers and Dr. J.W. Cook (MRC.56/543, CAMO.56/4 - Enclosure A).

      (b) by Professor F. Morton (to be circulated).

   (ii) Biological -

      (a) No.XVI (May 1956), tests completed by Dr. D.L. Woodhouse (1954-55), and tests commenced February 1956, (MRC.56/542, CAMC.56/3 - Enclosure B).

      (b) Statistical analysis of 1954-55 experiments by Dr. J.O. Irwin, (MRC.56/548, CAMO.56/5 - Enclosure D).

      (c) On 1954-55 experiments with mice at Leeds, by Dr. J.O. Irwin, (MRC.56/549, CAMC.56/6 - Enclosure E).

3. Discussion of Data tables of mineral oil fractions submitted by Dr. D.L. Woodhouse (section two of MRC.56/542, CAMO.56/3 - Enclosure C)

4. Future plans.

5. Any other business.

6. Date of next meeting.

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MRC.56/543
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COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

Report by W. Carruthers and J.W. Cook

The picrate-forming fractions K_1S_1aAp - K_4S_8bAp have been subdivided by chromatography on alumina into four main fractions:-

(i) eluted with petroleum ether, refractive index less than 1.5000. These fractions were generally solid at room temperature, and consisted mainly of waxy material,

(ii) eluted with petroleum ether, refractive index greater than 1.5000,

(iii) eluted with benzene,

(iv) eluted with methanol.

The table shows the fractionation obtained in the different experiments.

The petroleum ether eluates p (ii) were combined, and the composite material was carefully refractionated, using a spinning band fractionating column, in Professor Morton's laboratory. We are greatly indebted to Professor Morton for having this fractionation carried out for us. Thirty-one fractions and a residue were collected. We are now engaged in a sytematic examination of these fractions by crystallisation of molecular complexes and other means. A preliminary survey of their ultra-violet absorption spectra disclosed, disappointingly, that no separation of basic ring structures had been achieved by the distillation. The spectra of fractions over the whole range of the distillation were practically superimposable.

Chromatographic Separation of Picrate-forming Fractions from Kuwait Oil

Original Distillate		Extract (A)	Picrate-forming Fractions		Chromatography of Picrate-forming material				
Designation	b.p.	Vol. (c.c.)	Wt.(g.)	% of Extract	Wt. used	Fractions (g.) 1	2	3	4
K_4S_4a	365-367½	570	200	39	164	60	77	19	9.
K_4S_4b	367½-370	1130	302	30	243	103	103	25	9.
K_4S_5a	370-372½	790	194	27	153	63	90	9.6	6
K_4S_5b	372½-375	1150	225	22	188	33	120	24	13
K_4S_6a	375-377½	210	98	52	-	-	-	-	-
K_4S_6b	377½-380	1430	243	19	243*	33	193	-	175
K_4S_7a	380-382½	1000	280	31	245	54	157	22	15
K_4S_7b	382½-385	1100	280	28	260	80	131	27	21
K_4S_8a	385-387½	1050	207	22	180	42	102	20	13
K_4S_8b	387½-390	735	194	30	159	39	80	30	10

x The difference in these two series of figures is accounted for by the fact that some of the picrate-forming material was consumed in animal experiments.

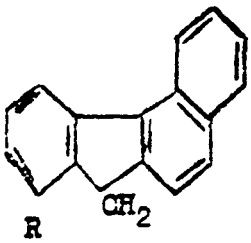
+ This fraction was chromatographed before withdrawing a quantity for the animal experiments.

The benzene eluates p (iii) were individually subjected to careful chromatographic analysis on alumina, and a number of crystalline compounds have thereby been isolated from them. Fraction K_4S_8bAp (iii) afforded a hydrocarbon m.p. 125-126° identified as 8-methyl-1:2-benzfluorene (I, R = CH_3) by comparison with an authentic specimen and by oxidation to the fluorenone. The parent hydrocarbon, 1:2-benzfluorene (I, R = H) was subsequently obtained from fractions K_4S_5bAp (iii) and K_4S_4aAp (iii) and was likewise identified by mixed melting point.

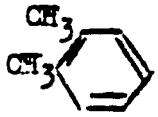
Chromatography of K_4S_7bAp (iii) yielded, among other products, a crystalline compound $C_{16}H_{16}S$, shown by its ultra-violet absorption spectrum to be a derivative of dibenzothiophene. Desulphurisation by boiling the compound with Raney nickel in ethanol afforded a hydrocarbon $C_{16}H_{18}$ identified as 3:4:3':4'-tetramethyldiphenyl (II). The sulphur compound is thus obviously a tetramethyldibenzothiophene for which there are only three possible alternative orientations. One of these has been eliminated by the synthesis of 2:3:6:7-tetramethyldibenzothiophene (III), which was not identical with the compound from the oil fraction.

Another dibenzothiophene derivative $C_{16}H_{16}S$ was found in the fraction K_4S_7aAp (iii), but as yet nothing is known of its more detailed structure.

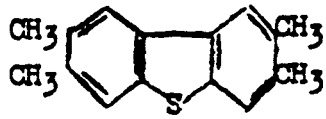
In addition to the above compounds, we also encountered, in several different fractions, 1:2:8-trimethylphenanthrene (IV) and 1-methylpyrene (V), both of which have been isolated before from other fractions of the Kuwait oil.



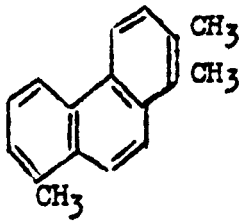
(I)



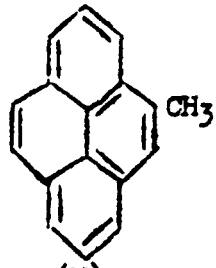
(II)



(III)



(IV)



(V)

May, 1956

University of Glasgow

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MRC.56/548
CAMO.56/5

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

Statistical Analysis of 1954-55 Experiments at Birmingham

by

J. O. Irwin

Experiments with 21 treatments were carried out with both mice and rabbits. The 18 principal fractions tested were the "m", "p" and "r" fractions derived from K_4S_4A - K_4S_8A. The a and b (lower and higher body) fractions of K_4S_6A were tested separately. In addition K_4S_9 was tested. This was a fraction boiling just above 390°C. prepared from the residue left after distillation of K_4S_1-8. Complete details of the nature and preparation of these fractions are given in CAMO.54/10,(MRC.54/606).

In addition the same two standards D(2) and D($\frac{1}{2}$) were used as before (i.e. 0.2% and 0.05% dimethyl 1:2 benzanthracene in liquid paraffin).

Experiments with Mice

The results are given in Table 1.

The Standard

The responses were very close to those obtained in previous years (particularly 1953-54), which are here inserted for comparison.

Standard		E.T.L. (52 weeks)	10% Date	Total number of tumours
D_2	1952-3	13.9 ± 0.4	11.4 ± 0.8	38
	3-4	12.9 ± 0.6	8.7 ± 0.4	40
	4-5	14.4 ± 2.0	6.6 ± 0.2	36
$D_{\frac{1}{2}}$	1952-3	24.8 ± 1.6	17.1 ± 0.6	25
	3-4	18.1 ± 0.8	10.3 ± 0.6	45
	4-5	17.9 ± 0.8	11.7 ± 0.3	43

The Fractions

The test with mice has not resulted in any discrimination between the fractions, the number of tumours is very small indeed. The results, which are included here for completeness, make it questionable whether these tests are worth continuing in future years.

Experiments with Rabbits

The design of this experiment was the same as that used in the previous two years (see M.R.C. 54/284, CAMO 54/2 and M.R.C. 54/887, CAMO 54/9); the 21 fractions were assigned randomly to the letters a, b, c, ...u,. The key is given in Table 2. There are 21 groups of 5 rabbits. In each group each rabbit

discussion as possibly providing a basis for the concise tabulation of data which would be convenient for the workers in the several centres and for committee members. They would complement the "Tree" prepared by Professor Morton. The sheets would need extension, etc. from time to time and would help in the decision as to what other fractions might need attention.

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COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

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was painted with the same 5 fractions, one on each of the sites I, II, III, [illegible] and VII. Each of the 21 sub-fractions or standards was painted on each sit in one and only one group. Each fraction was used in exactly 5 groups and every pair of fractions in just 1 group.

The percentage of each group of 5 animals which got tumours on each site was calculated. The method of analysis was to transform percentages to degrees by means of the angular transformation (which approximately equalises variance at different levels) and then to perform an analysis of variance of the results. Table 3 shows the mean values of the transformed variables and the probabilities of getting a tumour, estimated for each fraction and standard.

In a few instances recorded tumours were marked with a query; most of these were present at the end of the test, but it was doubtful whether or not they were actually papillomas. This never affected the number of tumours, out of 25 possible for any one fraction, by more than one (or in one case 2); e.g. for fraction K_4S_4Ap the lower result was 1/25 and the higher 2/25; for D_2 the lower result was 10/25 and the higher 11/25; for the K_4S_6Aap the lower result was 2 and the higher 4. The results were analysed both including and excluding the doubtful tumours. This made no appreciable difference, and here the results excluding the doubtful tumours are given.

The standards

The results are here compared with those of previous years:-

	1952-3	1953-4	1954-5
D_2	.798 (60.6)	.643 (52.2)	.776 (59.4)
$D_{\frac{1}{2}}$	.296 (34.6)	.156 (26.6)	.399 (39.9)
Standard Error	(5.0)	(5.0)	(4.7)

The estimated probability of a tumour is given above, and the transformed variable is shown in brackets. The results are in good agreement; there is no significant difference from year to year.

The Fractions

K_4S_9, curiously enough, appears to be the most carcinogenic. The m series is almost inert, the p series with the exception of K_4S6A_aP is only slightly active. K_4S6A_aP is significantly more active than the other p fractions; the r series is more active than the other p fractions; the difference just reaches the 5% level of significance. K_4S_9 is not significantly more active than K_4S6A_aP.

Analysis of Variance

The analysis of variance is shown in Table 4. There are significant differences between sites, but not this time between the different groups of rabbits. In 1953-4 the site effect was significant but the group effect was not. In 1952-3 both effects were significant. The combined evidence leaves no doubt that both effects are real.

The greatest number of tumours (see Table 5) is again on site VII (middle of the back nearest the ears).

Table 1

Experiments with mice at Birmingham in 1954-5

Standard or Fraction	Expectation of Tumourless Life (limited to 52 weeks)	Date when 10% of survivors had tumours	Total number of tumours
D_2	14.4 ± 2.0	6.6 ± 0.2	36
$D(\frac{1}{2})$	17.9 ± 0.8	11.7 ± 0.3	43
K_4S_9	50.8 ± 0.8	not reached	2
K_4S_4Am	51.7 ± 0.3	not reached	1
K_4S_5Am	52	not reached	0
$K_4S_6A_am$	52	not reached	0
$K_4S_6A_bm$	51.1 ± 0.7	not reached	2
K_4S_7Am	52	not reached	0
K_4S_4Ap	52	not reached	0
K_4S_5Ap	52	not reached	0
$K_4S_6A_ap$	52	not reached	0
$K_4S_6A_bp$	52	not reached	0
K_4S_7Ap	51.3 ± 0.5	47.5 ± 1.0	2
K_4S_8Ap	51.7 ± 0.4	not reached	1
K_4S_4Ar	51.2 ± 0.8	not reached	1
K_4S_5Ar	52	not reached	0
$K_4S_6A_ar$	51.1 ± 0.7	not reached	3
$K_4S_6A_br$	51.6 ± 0.3	not reached	2
K_4S_7Ar	51.1 ± 0.9	not reached	1
K_4S_8Ar	51.4 ± 0.4	not reached	2

The Carcinogenic Activity of the m, p, r series compared with the fractions from which they were derived

The dimethyl-benzanthracene equivalent of the fractions has been calculated on the assumption that the relation between response (measured by the transformed variable (y)) and the logarithm of concentration is linear and has the same slope for the fractions as for the standard. The relation is then of the form $Y = a + bx$ where $x = \log_{10}$ concentration. The slope was estimated from the values of y obtained for D_2 and $D_{\frac{1}{2}}$. The slope estimates in 1953-4 and in 1954-5 did not differ significantly and the mean of the two was used. Thus the equations of the concentration-response lines for the two years were estimated to be

1954-5 $\quad Y = 49.65 + 37.36x$

1953-4 $\quad Y = 39.41 + 37.36x$

By substituting for Y the experimental values obtained for any fraction, the corresponding value of x, and the corresponding "D equivalent" can be obtained.

The results are given in Table 6. The last column of Table 6 suggests that on the average the sum of the activities of the m, p, r fractions is only about 40% of that of the fractions from which they were derived.

No great reliance can be placed on the precise figures; at present we know little about the concentration-response curves for the fractions* so that the assumptions on which the calculation is based may be in error.

On the simple assumption (certainly not true) that the concentration of carcinogenic material is directly proportional to the percentage of tumour-bearing sites, the estimate of the original activity recovered in the m, p, r fractions is about 60%. Thus there has certainly been some loss of activity which may be of the order of 50%. The reasons for this deserve careful consideration.

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* The only direct evidence is from the dilution experiments carried out at Birmingham in 1949-50. This is favourable, as far as it goes; there was no significant difference between the slopes of the concentration-response curves for fractions J and F and the standard.

Table 2

Experiments with Rabbits at Birmingham 1954-5

Rabbits	Sites I	II	III	IV	VII
1 - 5	n	a	d	u	p
6 - 10	o	b	e	a	q
11 - 15	p	c	f	b	r
16 - 20	q	d	g	c	s
21 - 25	r	e	h	d	t
26 - 30	s	f	i	e	u
31 - 35	t	g	j	f	a
36 - 40	u	h	k	g	b
41 - 45	a	i	l	h	c
46 - 50	b	j	m	i	d
51 - 55	c	k	n	j	e
56 - 60	d	l	o	k	f
61 - 65	e	m	p	l	g
66 - 70	f	n	q	m	h
71 - 75	g	o	r	n	i
76 - 80	h	p	s	o	j
81 - 85	i	q	t	p	k
86 - 90	j	r	u	q	l
91 - 95	k	s	a	r	m
96 - 100	l	t	b	s	n
101 - 105	m	u	c	t	o

$K_4S_9A_1m$ = a	$K_4S_9A_1p$ = q	$K_4S_9A_1r$ = s	K_4S_9 = l
$K_4S_7A_1m$ = c	$K_4S_7A_1p$ = h	$K_4S_7A_1r$ = k	D(2) = u
$K_4S_6A_2m$ = f	$K_4S_6A_2p$ = g	$K_4S_6A_2r$ = b	$D(\frac{1}{2})$ = i
$K_4S_6A_1B_1m$ = m	$K_4S_6A_1B_1p$ = o	$K_4S_6A_1B_1r$ = c	
$K_4S_5A_1m$ = n	$K_4S_5A_1p$ = d	$K_4S_5A_1r$ = r	
$K_4S_1A_1m$ = j	$K_4S_1A_1p$ = t	$K_4S_1A_1r$ = p	

a = $K_4S_9A_1m$	g = $K_4S_6A_2p$	m = $K_4S_6A_1B_1m$	s = $K_4S_9A_1r$
b = $K_4S_6A_2r$	h = $K_4S_7A_1p$	n = $K_4S_5A_1m$	t = $K_4S_1A_1p$
c = $K_4S_6A_1B_1r$	i = $D(\frac{1}{2})$	o = $K_4S_7A_1m$	u = D(2)
d = $K_4S_5A_1p$	j = $K_4S_1A_1m$	p = $K_4S_1A_1r$	
e = $K_4S_6A_1B_1p$	k = $K_4S_7A_1r$	q = $K_4S_9A_1p$	
f = $K_4S_6A_2m$	l = K_4S_9	r = $K_4S_5A_1r$	

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Table 3

Experiments with Rabbits at Birmingham 1954-5

Two "standard" and nineteen derived fractions

Mean values corrected for group differences

Fraction or Standard	Transformed variable (°)	Probability of Tumour	Total number of Tumours (out of 25 possible).
K_4S_4Am	16.14	.014	0
K_4S_5Am	14.28	0	0
$K_4S_6A_am$	14.04	0	0
$K_4S_6A_bm$	18.76	.044	1
K_4S_7Am	21.67	.082	2
K_4S_8Am	17.95	.034	1
K_4S_4Ap	18.76	.044	1
K_4S_5Ap	22.26	.090	3
$K_4S_6A_ap$	36.79	.338	9
$K_4S_6A_bp$	20.11	.061	2
K_4S_7Ap	23.56	.109	3
K_4S_8Ap	27.76	.175	5
K_4S_4Ar	26.24	.150	5
K_4S_5Ar	30.32	.218	6
$K_4S_6A_ar$	29.94	.211	6
$K_4S_6A_br$	32.57	.258	7
K_4S_7Ar	23.57	.109	3
K_4S_8Ar	26.61	.156	5
K_4S_9	45.54	.511	13
D(2)	59.36	.776	19
D(1)	39.94	.399	10
S.E. common	4.72		
Diff. between two means	6.67		

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Table 4

Experiments with Rabbits at Birmingham 1954-5

Analysis of variance on angular transformation of proportion of tumours out of 5

	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio	
Sites	1944.52	4	486.13	4.64	$(.01 < P < .001)$
Groups of Rabbits	3090.86	20	154.54	1.47	n.s.
Fractions	13450.19	20	672.51	6.42	$(.01 < P < .001)$
Error	6288.09	60	104.80		
	24773.66				

Table 5

Experiments with Rabbits at Birmingham 1954-5

Number of tumours out of 105 possible on the five different sites

Site		Number of tumours
I	Ears	19
II	Ears	16
III		17
IV		14
VII	Middle of back nearest ears	35

Table 6

B. Dibenzanthracene Equivalents of 1954-5 fractions Compared with the fractions from which they were derived

	y	p	x	X	% Total	% corresponding fraction in 1953-4
1954-5						
K_4S_1Am	16.14	.014	$\bar{1}$.1031	.1268	24.8	6.0
p	18.76	.044	$\bar{1}$.1732	.1490	29.1	7.0
r	26.24	.150	$\bar{1}$.3734	.2362	46.1	11.2
		.208		.3120	100.0	24.2
K_4S_5Am	14.28	.005	$\bar{1}$.0533	.1131	18.8	6.6
p	22.26	.090	$\bar{1}$.2669	.1849	30.7	10.8
r	30.32	.218	$\bar{1}$.4826	.3038	50.5	19.2
		.313		.6018	100.0	36.6
K_4S_6Am	14.04	0	$\bar{1}$.0468	.1113	12.9	4.9
p	36.79	.338	$\bar{1}$.6558	.4527	52.6	19.9
r	29.94	.211	$\bar{1}$.4724	.2968	34.5	13.0
		.549		.8608	100.0	37.8
K_4S_6Am	18.76	.044	$\bar{1}$.1732	.1490	22.6	6.5
p	20.11	.061	$\bar{1}$.2093	.1619	24.5	7.1
r	32.57	.258	1.5428	.3490	52.9	15.3
		.363		.6599	100.0	28.9
K_4S_7Am	21.67	.082	$\bar{1}$.2511	.1783	30.8	19.0
p	23.56	.109	$\bar{1}$.3017	.2003	34.6	21.4
r	23.57	.109	$\bar{1}$.3019	.2004	34.6	21.4
		.300		.5790	100.0	61.8
K_4S_8Am	17.95	.034	$\bar{1}$.1515	.1417	22.0	7.7
p	27.76	.175	$\bar{1}$.4141	.2595	40.4	14.1
r	26.61	.156	$\bar{1}$.3833	.2417	37.6	13.1
		.365		.6429	100.0	34.9
1953-4						
K_4S_1A	51.57	.631	.3255	2.116		
K_4S_5A	48.14	.563	.2337	1.713		
K_4S_6A	52.77	.654	.3576	2.279		
K_4S_7A	38.35	.366	$\bar{1}$.9716	0.937		
K_4S_8A	49.35	.587	.2661	1.843		

p = probability of a tumour occurring

y = angular transform of p

X = "D equivalent"

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COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

Report on the 1954-5 Experiments with Mice at Leeds

by

J.O. Irwin

An experiment with 11 fractions (or standards) was carried out. These were:-

K_4S_2	(k)	K_4S_6	(c)	$K_{4.4}$	(f)
K_4S_3a	(a)	K_4S_6a	(d)	$K_{4.4}(\frac{1}{2})$	(g)
K_4S_4	(i)	K_4S_8b	(e)	$D(2)$	(j)
K_4S_5b	(b)			$D(\frac{1}{2})$	(h)

Fractions (k), (a), (b), (c), (d), (g), (i) produced no tumours. The results for the other fractions are given in Table 1, where certain comparisons are made with the 1953-4 results at Birmingham. That for K_4S_8b is based on the second tests for this fraction. The first was discarded because the animals suffered severe depletion by an outbreak of haemolytic streptococcal septicaemia.

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The Standards

The lower and higher concentrations are not very clearly differentiated; there were actually fewer tumours with $D(2)$ than with $D(\frac{1}{2})$ though the former gave a significantly earlier value of the date when 10% of survivors had tumours. K_4S_3a and $K_{4.4}(\frac{1}{2})$ yielded no tumours in these experiments; the same happened with K_4S_3 and $K_{4.4}(\frac{1}{2})$ in the 1953-4 experiments at Birmingham. However in the Birmingham experiments in 1953-4, K_4S_2, K_4S_5 and K_4S_6 yielded one or two tumours, whereas the corresponding fractions in the present experiments were entirely negative.

The results for $K_{4.4}$ and K_4S_8b are compared with the corresponding results at Birmingham in 1953-4. The Leeds results suggest slightly higher activity.

Table 1

Experiments with mice at Leeds 1954-5, and certain comparisons with the 1953-4 Birmingham results

Standard or Fraction	Expectation of Tumourless Life (limited to 52 weeks)	Date when 10% of survivors had tumours	Total number of tumours
Leeds 1954-5			
$D(2)$	16.1 ± 1.8	10.1 ± 0.5	41
$D(\frac{1}{2})$	18.8 ± 0.9	12.2 ± 0.2	44
$K_{4.4}$	46.9 ± 2.1	26.4 ± 1.0	5
K_4S_8b	49.0 ± 1.7	31.5 ± 1.1	3
Birmingham 1953-4			
$D(2)$	12.9 ± 0.6	8.7 ± 0.4	40
$D(\frac{1}{2})$	18.1 ± 1.0	10.3 ± 0.6	45
$K_{4.4}$	50.6 ± 0.8	not reached	2
K_4S_8	51.6 ± 0.4	not reached	1
K_4S_2	51.9 ± 0.2	not reached	1
K_4S_5	51.2 ± 0.8	not reached	1
K_4S_6	51.9 ± 0.1	not reached	1

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July 18, 1956

Messrs.: E. W. Adams, L. E. Curtis, C. H. Hine, E. K. Linder, R. C. Mithoff, M. E. Bouquist, A. C. Pabst

Gentlemen:

I thought each of you would be interested in the attached paper reproduced from volume 16, May 1956 of "Cancer Research."

This work contains results obtained by S. C. Brooks and Dr. Baumann on skin sterols. You will remember that our Subcommittee considered supporting Dr. Baumann's work in the past and I thought you would be interested in some of his results with petroleum products.

Very truly yours,

R. E. ECKARDT, M. D.

REE:ejh
Attach: Skin Sterols I. Studies with Certain Hyperplasia-inducing Hydrocarbons.
S. C. Brooks and C. A. Baumann.
CANCER RESEARCH 16:4 (May 1956)

cc - R. V. Stroup (w/attach.)

API 06185

Skin Sterols

V. Studies with Certain Hyperplasia-inducing Hydrocarbons*

S. C. Brooks and C. A. Baumann

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wis.

In previous studies (6, 7) the application of carcinogenic hydrocarbons to mice decreased the percentage of Δ^7-cholestenol in the sterol mixture of the separated epidermis, whereas noncarcinogens such as phenanthrene were either inactive or very much less effective. Mineral oils capable of inducing hyperkeratosis in calves or guinea pigs (4, 5) also lowered the percentage of Δ^7-cholestenol in the sterol mixture of mouse epidermis (7). Subsequent studies with fractions from mineral oil showed the activity to be present in many fractions including those consisting of simple straight-chain hydrocarbons. In the present study a series of hyperplasia-inducing hydrocarbons has been studied, and the results expressed in a way that differentiates true decreases in Δ^7-cholestenol from apparent decreases due to dilution with extra cholesterol.

MATERIALS AND METHODS

The compounds studied included saturated aliphatic hydrocarbons $C_{?}$ to $C_{?}$ in length, corresponding mono-unsaturated hydrocarbons, and certain relatively simple aromatic compounds such as n-butylbenzene and phenyldodecane. Comparisons were made with effects of carcinogens such as methylcholanthrene and benzpyrene, and with appropriate fractions of commercial mineral oil. The liquids were purified by distillation over a 2° range; the solids were used as purchased when the melting point was satisfactory.

Male albino mice of the Rockland Strain (Stokely-Peterson Co.), 7–10 weeks of age were maintained on commercial dog chow. The hair was removed with an electric shaver, and the compounds or solutions were applied quantitatively to the depilated area of the back with a tuberculin syringe. Two-tenths of a cc of the liquid or an appropriate solution was applied to each mouse on the 1st, 3d, and 5th days of the experiment. The skin was removed on the 6th day, the muscle scraped from the dermis with a scalpel, and, when indicated, the skin was [illegible] to 1.5 [illegible] tones. After being [illegible] in 0.35 N NH_4OH for [illegible] the epidermis was separated from the dermis with a [illegible] scalpel. The materials from pairs of mice were then analyzed for sterols by the methods described previously (6, 12, 17).

The problem of expressing results.—In previous studies results were expressed either as the percentage of fast-acting sterols in the sterol mixture of the epidermis or as mg of sterol per unit of fat-free dry weight (6–8, 17). In this sense methylcholanthrene and related carcinogens as well as certain mineral oils appeared to decrease the concentration of Δ^7-cholestenol in mouse epidermis. Since, however, these agents also caused a hyperplasia as indicated by an increased percentage of dry fat-free epidermis per unit weight of dry fat-free whole skin, it was not clear whether the decrease in the proportion of Δ^7-cholestenol in the epidermis represented a true decrease in this sterol or whether it was the result of a dilution of normal epidermis with cells or material low in this sterol (Chart 1). The expression "mg sterol/gm epidermis" is a fraction, the numerical value of which can be lowered either by decreasing the numerator or by increasing the denominator, and in hyperplasia the denominator may have been increased as much as fourfold. Thus, when hyperplasia is as rapid and marked as in the present studies, the true changes in epidermal sterols are probably revealed more clearly by expressing the results on the basis of skin area than on so variable a basis as the weight of the epidermis itself.

Control groups from twelve series of experiments (Table 1) showed the fat-free dry dermis of untreated mice to weigh 12.4 ± 1.7 mg/sq cm and the epidermis to weigh 2.5 ± [illegible] mg. A tabulation (3) of the results of 60 experimental treatments showed the epidermis to increase in weight to variable degrees up to 315 per cent of the controls, whereas the dermis was relatively constant, the maximum increase being 50 per cent when hyperplasia was extreme. Without exception, however, the increases within the dermis, when they occurred at all, were so much smaller than the corresponding increases in the epidermis that, for all practical purposes, dermal weight could be regarded as a measure of skin area. Data from earlier series in which the skin was not cut into constant areas were, therefore, converted to an area basis by means of the average weight of 1 sq cm of dermis.

RESULTS

Effect of methylcholanthrene (MC) and other carcinogens on skin sterols.—The minimum concentration of MC in acetone to produce a hyperplasia was 0.025 per cent, at which dosage an increase in cholesterol was barely detectable. Higher concentrations of MC caused increases in both cholesterol and in epidermal weight with a simultaneous de-

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Most of these results are contained in a thesis entitled "Contrasting Effects of Certain Organic Compounds on Skin Sterols and on the Epidermis" by S. C. Brooks, University of Wisconsin, June, 1955, in which the experimental procedures and the variations in results are recorded in considerable detail.

Received for publication December 14, 1955.

crease in the concentration of Δ^7-cholestenol (Table 2). The maximum decrease occurred when 0.2 per cent of MC was applied; 0.3 per cent caused a heavy hyperplasia and a greater increase in cholesterol, but without as great a decrease in Δ^7-cholestenol, possibly because of a rapid scabbing which may have decreased the penetration of subsequent applications of MC into the lower layers of the epidermis.

CHART 1

Chart 2 illustrates the changing concentrations of the two sterols with increasing hyperplasia due to MC. The increases in cholesterol paralleled and slightly exceeded the increases in fat-free dry epidermal weight, whereas the decrease in Δ^7-cholestenol was substantial. Thus, the previously observed decreases in Δ^7-cholestenol in the sterol mixture from MC-treated skin (6, 7) appear to have been the result of two processes, a true decrease in the amount present per unit of area, aggravated by dilution with extra cholesterol.

Comparative data of five other compounds of

TABLE 1

STEROLS AND EPIDERMAL WEIGHT OF NORMAL MOUSE SKIN

Series*	μg Epidermal cholesterol/ sq cm	μg Epidermal Δ^7-cholestenol/ sq cm	Mg. dermus† sq. cm.	Mg. epidermis† sq. cm.
III‡	56.0	48.0		
V‡	58.0	42.0		
VI‡	54.5	53.0		
VII‡	46.0	60.5		
IX	46.0	43.5	12.3	3.2
X	56.0	30.5	13.6	3.5
XI	57.0	49.5	12.3	2.6
XII	35.5	50.0	10.1	1.9
XIII	30.2	61.0	15.0	4.5
XIV	47.5	49.5	9.9	2.1
XVI	46.5	63.5	12.3	2.4
XVII	47.5	47.0	12.6	3.2
Mean	51.6±11.4	52.2± 9.2	12.4± 1.7	2.9±0.9

* All series include two pools of two mice each except series III (four pools) and VI and VII (one each).
Mean ± standard deviation.
† Dry acetone-extracted residue.
‡ Area was calculated from dermal weight.

TABLE 2

EFFECT OF CERTAIN CARCINOGENS ON SKIN STEROLS AND ON EPIDERMAL WEIGHT

Series	Compound* applied in acetone (per cent)	Mg.† epidermis/ sq cm	μg Epidermal cholesterol/ sq cm	μg Epidermal Δ^7-cholestenol/ sq cm	$\frac{\Delta^7 \times 100}{\text{total sterols}}$ (per cent)	No. samples
III-XVII	None	2.9±0.9	51.6±11.4	52.2±9.2	50	25
IX	None	2.9	52.0	54.5	51	2
IX‡	0.0167 MC	3.3	42.5	42.0	50	2
IX‡	0.025 MC	5.0	62.0	44.5	41	2
XI	0.05 MC	4.5	102.0	27.5	20	2
VI‡, IX	0.1 MC	4.6	91.0	19.0	17	4
IV‡, IX, X	0.2 MC	4.6	96.5	13.5	11	5
IX	0.3 MC	7.5	170.5	34.5	17	2
XVI	None	2.4	48.5	63.5	56	2
XVI	0.2 MC	4.9	111.5	26.5	19	2
XVI	0.2 BP	4.8	106.5	20.5	22	2
XVI	0.2 DMBA	4.5	106.0	39.5	27	4
XVI	0.2 DBA	3.9	78.0	30.0	33	3
XVI	0.2 BA	4.8	104.0	26.0	20	2
XVI	4.0 Croton oil	10.3	160.0	62.5	28	2

* MC = Methylcholanthrene.
BP = Benzopyrene.
DMBA = 7,12-dimethylbenz(a)anthracene.
DBA = Dibenzanthracene.
BA = Benzanthracene.

† Dry acetone-extracted residue.
‡ Area was calculated from dermal weight.

various carcinogenic strengths are presented in Table 2. A true lowering of Δ^7-cholestenol was exhibited by 0.2 per cent solutions of methylcholanthrene, benz(*a*)pyrene, 7,12-dimethylbenz(*a*)-anthracene, and 1,2,5,6-dibenzanthracene, whereas two weakly carcinogenic solutions, 0.3 per cent of 1,2-benzanthracene and 4.0 per cent of croton oil, showed no lowering of Δ^7-cholestenol in this 6-day test. Each of these compounds produced hyperplasia and a corresponding increase in the amount of epidermal cholesterol.

Effect of methylcholanthrene in certain mineral oils.—Others have shown that the carcinogenicity of MC varies with the vehicle in which it is dissolved (2, 14, 15). In the present study, 0.2 per cent of MC was applied in mineral oils of widely different viscosities, a very heavy pharmaceutical USP mineral oil and a much lighter oil, "Eureka," oil 4.[1] The changes induced in the skin sterols were panied by only a minimal hyperplasia and a minimal increase in cholesterol (Table 3).

A partial explanation for the reduced effective-

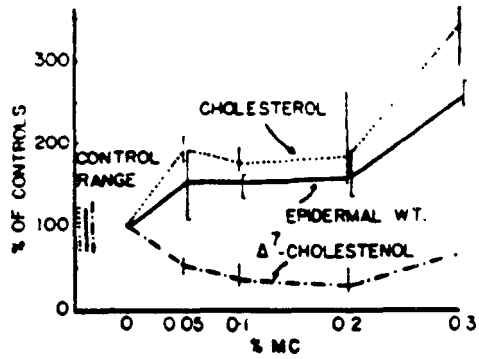

CHART 2.—Changes in skin sterols and epidermal weight with the application of various levels of methylcholanthrene in acetone. Narrow lines represent ranges.

TABLE 3

ALTERED EFFECTIVENESS OF METHYLCHOLANTHRENE IN MINERAL OIL

Series	Application	Mg* epidermis/ sq cm	μg Epidermal cholesterol/ sq cm	μg Epidermal Δ^7-cholestenol/ sq cm	$\frac{\Delta^7 \times 100}{\text{total sterols}}$ (per cent)	No. samples
III–XVII	None	2 9±0 9	51 6±11 4	52 2±9.2	50	25
V†	USP mineral oil	3 0	54 5	50 5	49	2
VI†	"Eureka" mineral oil	4 2	72 5	59 5	46	2
V†	0 2 per cent MC in USP mineral oil	3 0	63 5	50 5	44	2
VI†	0 2 per cent MC in "Eureka" mineral oil	3 9	71 5	52.0	42	2
VI†, XIII	0 5 per cent MC in USP mineral oil	3 6	62.0	28 0	27	4

* Dry acetone-extracted residue.
† Area calculated from dermal weight.

much less when the carcinogen was applied in these oils (Table 3) than in acetone (Table 2): 0.2 per cent MC failed to alter the concentration of Δ^7-cholestenol from that observed when the oils were applied in the absence of carcinogen, whereas 0.5 per cent of MC in USP mineral oil caused a substantial decrease in Δ^7-cholestenol accom-

[1] The mineral oils and their fractions were obtained from Dr W. G. Hoekstra of this department; they were originally supplied by Dr G. W. Flint of the Standard Oil Co., Whiting, Indiana. Data on the oils themselves have been published previously (7).

The aromatic fraction of "Whiting" oil was prepared by silica gel chromatography. The n-paraffin fraction from the oil was prepared by urea adduction of the dearomatized oil. The iso-paraffin-rich fraction was prepared by thermal diffusion of the oil after removal of n-paraffins and aromatics: N_D^{20} range from 1.4688 to 1.4807. The naphthene-rich fraction was prepared by thermal diffusion of the oil after removal of n-paraffins and aromatics: N_D^{20} range from 1.4681 to 1.4749. The mono- and polycyclic aromatics were prepared from the aromatic fraction by thermal diffusion. The fraction rich in ring compounds was prepared by removing the n-paraffins from whole oil with urea, and iso-paraffins with thiourea.

ness of MC in mineral oil probably lies in a diminished penetration of carcinogen into the skin, with a substantial fraction remaining on the surface in the oil itself. However, the various effects of MC in mineral oil (Table 3) differed qualitatively from those observed when minimal amounts of MC were applied in acetone (Table 2), and hence it is suggested that the route of penetration of carcinogen into the skin probably varies with the solvent in which it is applied.

Homologous series of hydrocarbons.—Saturated hydrocarbons from octane to octadecane, and unsaturated analogs from 1-heptene to 1-octadecene were applied as the undiluted liquids. Virtually all the compounds in the two series proved to be hyperplastic agents, with the maximum effect observed when the chain was 14 carbon atoms long (Table 4). The amounts of epidermal cholesterol tended to parallel the increases in fat-free epidermal dry weight; the maximum increase in epidermal cholesterol in the saturated series was

1?8 per cent [illegible] as compared with an increase of [illegible] per cent under the influence of 1-tetradecene. In contrast to these changes in cholesterol, the concentrations of Δ⁷-cholestenol per unit area of hyperplastic epidermis were remarkably uniform, except for a slight increase with the application of the shorter-chain compounds. This constancy of Δ⁷-cholestenol in hyperplasia due to simple hydrocarbons was particularly apparent when the results were expressed as percentages of the normal values (Chart 3). The decrease in the percentage of Δ⁷-cholestenol in the sterol mixture (Table 4, column 6, and Ref. 7) induced by these hydrocarbons is therefore the arithmetical result of the dilution of a constant amount of Δ⁷-cholestenol with an increasing amount of cholesterol.

Aromatics and alkylated aromatics.—Most of the examples of this type of compound tested caused extreme hyperplasia after only three applications (Table 4), and the increases in cholesterol were also very large, e.g., over 500 per cent for *n*-butylbenzene. However, in spite of the magnitude of these effects, the changes in Δ⁷-cholestenol were minimal, and for two of the compounds, *n*-butylbenzene and phenyldodecane, the amount of Δ⁷-cholestenol was unchanged. The application of

TABLE 4

EFFECT OF CERTAIN ALIPHATIC AND AROMATIC HYDROCARBONS ON SKIN STEROLS AND ON EPIDERMAL WEIGHT

Series	Application	Mg* epidermis/sq. cm	µg Epidermal cholesterol/sq. cm	µg Epidermal Δ⁷-cholestenol/sq. cm	Δ⁷ × 100 / total sterols (per cent)	No. samples
III-XVII	None	2.9±0.9	51.6±11.4	52.2±9.2	50	25
XVII	Octane (Skelly F)	3.3	39.5	74.0	65	3
XVII	Undecane	4.5	55.5	52.5	34	4
VII†, XIV	Dodecane	5.0	91.0	45.5	33	4
VII†, XIV	Tetradecane	7.4	132.0	59.5	31	4
VI†, VII† XIV	Hexadecane	6.8	113.5	44.5	28	6
VII†, XIV	Octadecane	5.8	115.0	47.9	29	4
XVII	1-Heptene	4.9	93.5	71.0	43	3
XVII	1-Octene	6.5	125.0	78.5	39	4
XVII	1-Decene	9.4	181.0	50.5	22	3
X, XIV	1-Dodecene	7.1	129.5	60.5	30	3
X, XIV	1-Tetradecene	10.7	183.0	46.5	21	4
X, XIV	1-Hexadecene	6.6	118.0	39.0	27	4
X, XIV	1-Octadecene	5.6	103.0	46.0	30	4
XII	Ether	2.8	45.5	39.0	47	1
XII	Benzene	3.6	87.5	70.0	45	1
IX†	*p*-Dicyclohexylbenzene in ether	2.6	54.5	38.0	41	2
IX†, XIII, XVI	Cyclohexylbenzene	10.8	204.5	31.0	14	3
VII†, X, XIII, XVI	Phenyldodecane	11.2	146.5	54.5	27	7
X, XII	*n*-Butylbenzene	14.4	324.5	47.5	13	3
XII	Chrysene in benzene	11.5	194.5	43.5	18	2

* Dry acetone-extracted residue.

† Area calculated from dermal weight.

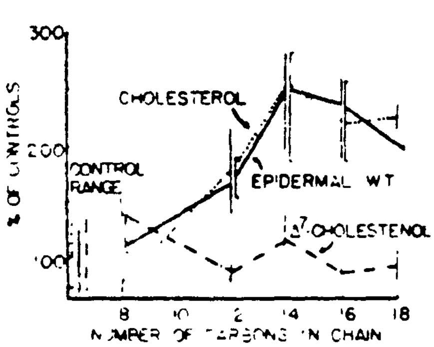

CHART 3.—Changes in skin sterols and epidermal weight with the application of a saturated homologous series of hydrocarbons. Narrow [illegible].

cyclohexylbenzene, on the other hand, caused a significant decrease in the Δ⁷-sterol, the over-all effect in the 6-day test resembling that observed when MC is applied in acetone.

Chrysene in benzene (30 mg/day) caused the cholesterol concentration to increase along with the epidermal weight, with no significant effect on Δ⁷-cholestenol. Benzene itself had no effect on the sterols or on epidermal weight.

Effect of hydrocarbons on esterification of skin sterols.—In normal mouse epidermis approximately 10 per cent of the Δ⁷-cholestenol and 74 per cent of the cholesterol was unesterified; simple hyperplasia had no effect on the esterification of either sterol (free to total ratio). An increased epidermal weight of as much as 172 per cent with the application of tetradecane did not change the esterification of the sterols. However, compounds which

API 06189

[illegible] caused increases in the proportion of the free sterols [illegible] epidermis [illegible] free fast-acting sterols [illegible] from [illegible] per cent to 55 per cent with [illegible] 32 per cent with [illegible] tetradecene.

Alkyl [illegible] aromatics also affected the esterification of [illegible] sterols and the increase in the percentage [illegible] cholesterol appeared to vary with the length [illegible] chain on the compound applied. Benzene [illegible] no effect, but n-butylbenzene, [illegible] benzene, and phenyldodecane increased the percentage of free Δ^7-cholestenol in epidermis. The application of chrysene also seemed to increase the proportion of free Δ^7-cholestenol. None of [illegible] sterol [illegible] the epidermis was esterified after the application of 1-tetradecene, phenyldodecane, or [illegible]sene. The other compounds exerted no effect or caused lesser increases in free cholesterol.

Effect of hydrocarbons on the dermis.—Approximately 65 per cent of the cholesterol and 12 per cent of the Δ^7-cholestenol in the skin was found in the "dermis" preparations. It is possible that this latter represents hair follicles and sebaceous glands which failed to separate completely from the dermis. The application of the various hyperplasia-inducing compounds failed to affect the cholesterol content of the dermis, unless the hyperplasia was so severe that there was also an increase in the dermal weight. In these cases the increase in cholesterol was somewhat greater than the increase in dermal weight.

Effect of fractions from certain mineral oils.—Kandutsch and Baumann (7) showed that certain mineral oils depressed the percentage of fast-acting sterols in the total sterol of treated epidermis. The Δ^7-cholestenol-lowering capacity and the hyperplastic potency of the oils appeared to depend on [illegible] content of [illegible] components [illegible] oil. In the present study an oil called "Midcontinent" [illegible] 7 [illegible] mineral seal oil, "Whiting," oil 3, and various fractions of oil 3 were applied to the skin as undiluted liquids. The "Midcontinent" oil (86.4 per cent unsulfonatable residue, boiling range 486–683° F) caused the greatest increase in cholesterol and in epidermal weight, plus a slight lowering of Δ^7-cholestenol, whereas the "Whiting" oil (91.5 per cent unsulfonatable residue, boiling range 510°–665° F) caused hyperplasia with an increase [illegible] cholesterol but no apparent lowering of Δ^7-cholestenol (Table 5).

Neither the n-paraffin fraction of "Whiting" oil (approximately 17 per cent of the whole oil) nor the *iso*-paraffin fraction (approximately 30 per cent) exerted any effect on the fast-acting sterols although both caused a hyperplasia and an increase in cholesterol. These fractions therefore reacted very much like the aliphatic hydrocarbons described in a previous section of this paper.

TABLE 5

EFFECT OF CERTAIN MINERAL OILS AND MINERAL OIL FRACTIONS ON SKIN STEROLS AND ON EPIDERMAL WEIGHT

Series	Application	Mg* epidermis/sq cm	μg Epidermal cholesterol/sq cm	μg Epidermal Δ^7-cholestenol/sq cm	$\Delta^7 \times 100$ / Total sterols per cent	No. samples
II-XV†	None	2.9±0.9	51.6±11.4	52.2±9.2	50	25
III-IV†	Midcontinent mineral oil‡	6.7	146.0	30.5	18	4
III†	Whiting mineral oil‡	5.7	123.0	50.5	29	2
VIII†	n-Paraffins‡	5.8	120.0	80.5	41	1
VIII†	iso-Paraffins‡	5.4	104.5	58.0	36	1
VIII†	Ring compounds‡	6.9	131.5	34.0	20	1
III†	Naphthenes‡	3.6	54.5	9.5	15	1
VII†	Monocyclic aromatics‡	9.8	175.0	24.0	12	1
VII†	Polycyclic aromatics‡	6.4	133.0	23.0	15	1

* Dry acetone-extracted residue.
† Area calculated from dermal weight.
‡ See footnote (text).

The "ring compound" fraction, on the other hand, caused a slight lowering of the fast-acting sterols accompanied by an increase in cholesterol and in epidermal weight. Similar effects were noted when the mono- and polycyclic aromatic fractions of the "ring compounds" were applied. A very great decrease in the fast-acting sterols resulted from the application of the "naphthene" fraction (approximately 30 per cent of the whole oil). Both aromatic fractions (approximately 17 per cent of whole oil combined) induced hyperplasia and increased the content of cholesterol, but the naphthenes had no effect on either epidermal weight or on cholesterol. Thus, the effect of the nonhyperplastic, Δ^7-cholestenol-lowering naphthene fraction was very similar to that observed when 0.5 per cent of MC was applied in USP mineral oil (Table 3).

DISCUSSION

In the light of the present results, it is still possible that changes in skin sterols may be useful for a rapid preliminary recognition of active carcinogens for skin (7). However, the percentage of fast-acting sterols in epidermis is not a reliable measurement for this purpose, since so many noncarcinogens lower this percentage by increasing the amount of cholesterol in epidermis. On the other hand, carcinogens consistently lowered the amount of Δ^7-cholestenol per unit area of skin. Since Δ^7-cholestenol appears to be associated with the sebaceous glands (see below), the value of the sterol test may depend ultimately upon whether a rapid destruction of sebaceous glands is a reliable test for skin carcinogens.

Three types of response were encountered in this study. The first was characterized by parallel increases in epidermal weight and cholesterol and by a relative constancy in Δ^7-cholestenol. This hyperplasia was caused by noncarcinogenic hydrocarbons such as the saturated alkanes and the longer-chained mono-unsaturated alkenes. The alkylated aromatics tested and some of the shorter alkenes produced similar but somewhat more intense effects, so that the dermis was also affected. The second type of response was shown by carcinogens administered in acetone. These solutions caused hyperplasia of the epidermis and also some increase in the weight of the dermis. Chemically, the hyperplasia was characterized by a marked decrease in Δ^7-cholestenol, plus an increase in cholesterol which tended to exceed the increases in epidermal weight. The third type of response was the comparatively rare one in which follicular structures were attacked without any hyperplasia of the epidermis proper nor of the dermis; this was observed after the application of MC in a heavy oil, or of the naphthene fraction from certain mineral oils. Chemically, this response was characterized by a decrease in the amount of Δ^7-cholestenol per unit area, with only minimal changes in cholesterol and in epidermal weight.

Some of the differences between these hyperplasias may have been due to specific sensitivities of different parts of the epidermis to the various compounds applied. The differences probably also reflect the ease and the route by which the hyperplastic agents penetrate the various parts of the epidermis. The pertinent theoretical possibilities are (*a*) direct percutaneous absorption; (*b*) transfollicular absorption (11) with subsequent diffusion into the rest of the epidermis, or a combination of follicular absorption and percutaneous absorption, and (*c*) absorption by the follicular structures without any substantial diffusion into the rest of the epidermis.

Many of the present results are consistent with the assumption that Δ^7-cholestenol is concentrated in the sebaceous glands. In the many examples of "type I" hyperplasia encountered, the increases in epidermal weight were apparently due to increases in ordinary epidermal substance, the new material containing about the same proportion of cholesterol as that originally present. However, this new material contained no Δ^7-cholestenol. Thus, compounds such as tetradecane appear to affect only the "pavement" part of the epidermis and leave the follicular structures unchanged. This effect would most probably result from percutaneous absorption.

When MC is applied in acetone, histological changes in the sebaceous glands are detectable after 1 day, and the number of sebaceous glands remaining after 5 days is very small (10). This rapid disappearance of the sebaceous glands parallels the rapid disappearance of Δ^7-cholestenol from the epidermis (Table 2 and Ref. 7). It also parallels the loss of lipase from the skin of mice treated with MC (10). This enzyme is normally concentrated in the sebaceous glands (10), and the sterol located in the sebaceous glands is said to be completely esterified (13, 16). The Δ^7-cholestenol found in epidermis is largely in the ester form. Further circumstantial evidence for the location of Δ^7-cholestenol in the sebaceous glands is the relative absence of the sterol from the skin of newborn rats and mice (8), before the glands have developed, and its presence in high amount in the skin of hairless mice which contain many such glands (9). It is also present in sebaceous cysts (9).

Accordingly, "type II hyperplasia," that due to MC and related carcinogens in acetone, can be visualized as resulting from transfollicular absorption of the carcinogen, with destruction of the sebaceous glands and of Δ^7-cholestenol, plus an increase in the epidermis proper. "Type III hyperplasia," that due to MC in mineral oil, on the other hand, appears to involve only the follicular structures.

SUMMARY

1. A series of hydrocarbons was applied to mouse skin, and the hyperplastic responses, as well as the changes in skin sterols were measured. Most of the compounds increased the weights of the epidermis without any significant change in the dermis.

2. Methylcholanthrene (MC) in acetone caused a hyperplasia and a marked increase in cholesterol

with a simultaneous decrease in Δ^7-cholestenol. Related carcinogens produced similar effects. MC [illegible] lowered the concentration of Δ^7-cholestenol without any appreciable hyperplasia or increase in cholesterol.

3. Straight-chain saturated hydrocarbons caused hyperplasias which reached a maximum with tetradecane. These compounds failed to alter the amount of Δ^7-cholestenol present, but substantial increases in cholesterol paralleled the increases in epidermal weight. Effects due to the monounsaturated hydrocarbons were quite similar to those due to the saturated hydrocarbons, with similar effects on the skin sterols.

4. While benzene had no effect on skin, several of its alkylated derivatives caused marked hyperplasias with parallel increases in cholesterol. But the amount of Δ^7-cholestenol in the epidermis remained unchanged, except after the application of cyclohexylbenzene, which caused it to decrease.

5. Hyperplasia involving an increase in epidermal weight of over 200 per cent was accompanied by an increase in unesterified Δ^7-cholestenol and in some cases by an increase in unesterified cholesterol.

6. Mineral oil fractions produced effects similar to those due to pure aliphatic or aromatic compounds of similar structure. The so-called naphthene-rich fraction lowered Δ^7-cholestenol, without causing hyperplasia.

7. The evidence available suggests that the Δ^7-cholestenol of normal mouse epidermis is located in the sebaceous glands.

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August 3, 1956

Dr. Robert A. Kehoe
University of Cincinnati
The Kettering Laboratory
College of Medicine - Eden Avenue
Cincinnati 19, Ohio

Dear Dr. Kehoe:

This constitutes a somewhat belated additional reply to your letter of June 2, and my letter to you of June 20.

The Subcommittee on Carcinogenicity has voted to continue the epidemiological studies at the Kettering Laboratories until January 1, 1957, in order that you might analyze the data which has been collected up until now and prepare a final report of the work for the benefit of the subcommittee and the Medical Advisory Committee. It was the feeling of the subcommittee that this could probably be done at an expense not to exceed $7,500.

Discussing this with Dr. Curtis by phone last Friday, he informed me that I, as chairman of the subcommittee, could proceed immediately, directly with you, in the negotiations to affect a continuation of these studies until January 1, 1957. Although it is already past the July 1, I think that you will understand the difficulties of getting opinions from diversely scattered members of the subcommittee and that this has occasion to delay in authorizing you to proceed with the work outlined in your letter to me. I trust that it will be possible to proceed, as you outlined, and to have Dr. Fhair analyze the data and prepare a final report for the MAC by the date of January 1, 1957.

By carbon copy of this letter to Mr. Stroop, authorization for expenses not to exceed $7,500 for this work is provided. It is understood that this money is already available in the budget of the subcommittee so that

it requires only authorization of the subcommittee to proceed with this expenditure.

If there are any questions for discussion perhaps these could be brought up at the next meeting of the subcommittee, but if they cannot wait until then, I would be glad to discuss them with you at any time.

Sincerely yours,

R. E. Eckardt

R. E. ECKARDT, M. D.

REE:cjh
cc - Messrs.: E. W. Adams
L. E. Curtis
C. H. Hine
E. K. Linder
R. C. Mithoff
M. N. Newquist
A. C. Pabst
J. J. Phair
D. V. Stroop

Carcinogenicity

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August 22, 1956

Mr. David V. Stroop
American Petroleum Institute
50 West 50th Street
New York 20, New York

Dear Mr. Stroop:

The attached article appeared in the NEW YORK TIMES on the morning of August 21, 1956.

Of particular interest to the petroleum industry is the section underlined in red. Although I do not agree with the report when it says that paraffines on milk cartons have produced cancer in man and experimental animals, nonetheless, it is likely that the various petroleum companies may receive inquiries as a result of this news item. In order to prepare them for this, I believe that the attached article should be circulated to all members of the Medical Advisory Committee of the API. Dr. Horton should be asked to prepare some data which provides information on this particular subject.

Very truly yours,

R. E. Eckardt

R. E. ECKARDT, M. D.

REE:cjh
Attach. - Article in THE NEW YORK TIMES, Tuesday, August 21, 1956. Cancer is Traced to Food Additives.

Copy From D. V. Stroop For Information Of Members Of Medical Advisory Committee and Technical Advisors
8/23/56

API 06195

The New York Times

TUESDAY, AUGUST 21, 1956. 31 L

CANCER IS TRACED TO FOOD ADDITIVES

Experts' Symposium in Rome Lists Many Dyes, Flavors, Preservatives as Unsafe

ASKS LEGISLATIVE CURB

Appeals for International Collaboration to Protect Mankind From Hazards

By ARNALDO CORTESI
Special to The New York Times

ROME, Aug. 20—A number of food additives used in the United States and Europe as dyes, thickeners, sweeteners preservatives and the like were labeled cancer-producing today by a symposium of the International Union Against Cancer.

Other food additives were put on a suspect list as unsafe until their properties had been more thoroughly tested.

The cancer experts meeting in Rome acknowledged that this created a "serious public health problem." They unanimously recognized the "urgent necessity of international collaboration for the protection of mankind" against such hazards as cancer-producing food additives.

The participants in the symposium also acknowledged that food additives were only one part of the vast problem of environmental cancer which includes occupational and lung cancers.

The symposium was attended by forty-two cancer experts from twenty-one countries, including seven Americans and four Russians. It was called by the congress of the International Union Against Cancer held in São Paulo, Brazil, in 1954.

Report Subject to Review

The joint report unanimously adopted by the symposium is subject to review by the Executive Committee of the International Union Against Cancer. It is, therefore, possible that some of its conclusions may be modified.

The report laid down the basic principle that no food additives should be used unless specifically permitted by legislation based on lists of substances that have been proved innocuous after stringent laboratory tests.

It went on to give the first lists of food preservatives and dyes that were found either acceptable, dubious or definitely dangerous. Dubious preservatives requiring urgent retesting included ethyl and butyl esters, thiodipropionic acid, sulphureous acid and its derivatives and formic acid.

The dangerous preservatives condemned as "carcinogenous and to be avoided for human use" included thiourea, tiocetamide, 8-hydroxyquinoline and hydroquinone. Most and perhaps all of these are used in the United States and Europe.

The report said certain mineral oils and paraffines used for coating milk containers had produced cancer in skin and experimental animals. It issued a warning against foodstuffs sterilized by radiation as potential cancer hazards and against the use of estrogens as fattening agents for poultry and meat animals.

It said that several detergents had "co-carcinogenous and prompting effects" and that their use for cleaning food containers therefore required caution.

Food Dyes Condemned

Food dyes came in for particularly severe condemnation from the symposium. Its report stated that no food dye at present met "agreed criteria of safety." Twenty-nine dyes were listed as "unsuitable" or "potentially dangerous" with the statement that they should on no account "be added to food or drink for men or animals."

Another list contained twenty-three dyes that might prove satisfactory after further tests.

The fundamental paper that formed the basis of the symposium's recommendations was read by Dr. Wilhelm C. Heuper, German-born member of the United States delegation. He is chief of the Environmental Cancer Section of the National Cancer Institute and is co-chairman of the symposium.

Dr. Heuper listed twenty groups of suspect food additives and seventeen groups of suspect food contaminants. Many of these agents, he said, have not been adequately investigated for carcinogenic qualities. The food additives included dyes, thickeners, synthetic sweeteners and flavors, preservatives, shortening, bleaches, oils and fat substitutes. The food contaminants included antibiotics and estrogen for fattening animals, pesticide residues, soot, chemical sterilizers, antisprouting agents, wrapping materials, radiation.

At the end of their labors the symposium and Executive Committee of International Union Against Cancer were received in audience by the Pope at his summer residence of Castel Gandolfo. He delivered a brief address and imparted an apostolic blessing to them and their work.

Ban on Three Dyes Upheld

More than two years ago, in the United States, the Federal Food and Drug Administration prohibited the use of three coal tar dyes on the ground that they were potentially harmful. Ten days ago the United States Court of Appeals upheld the ban on Orange No. 1, Orange No 2 and Red No 32

Copy From D. V. Stroop For Information Of Members Of Medical Advisory Committee & Technical Advisor 9/12/56

PUBLIC HEALTH COMMITTEE of the
PAPER CUP AND CONTAINER INSTITUTE
250 Park Avenue, New York 17, N.Y.

September 5, 1956

TO: ALL MEMBER COMPANIES

FROM: Homer N. Calver, Secretary

With further reference to my recent memoranda on the Rome Conference, you will be interested in the following quotations from two papers which were presented at the Joint Industrial Health Conference of the American Association of Physicians and Surgeons and the American Industrial Hygiene Association held in Atlantic City, New Jersey on April 25th, 1951:

"Unrefined 'slack waxes' induced occasional benign papillomas and a few cancers after prolonged painting. Aromatic extracts of such waxes were, however, much more active. Therefore, the carcinogenicity of 'slack waxes' is attributed not to the paraffins but to aromatic components of the oils from which they were pressed. The aromatic components are removed in the further purification of waxes for marketing."

From--Experimental Analysis of the Carcinogenic Activity of Certain Petroleum Products by William E. Smith, M.D. Douglas A. Sunderland, M.D. and Kanematsu Sugiura, ScD. New York.

"It has been found that certain crude waxes('slack waxes') have some carcinogenic activity. This was shown to be due to occlusion of minor amounts of the higher aromatics at intermediate stages of production. The drastic sulfuric acid treatment leading to the highly refined paraffin waxes, familiar in daily life, removes the aromatic contaminants to yield inert products."

From--Properties of High-Boiling Petroleum Products Physical and Chemical Properties as Related to Carcinogenic Activity, by H.G.M. Fischer, E.E. William Priestley, Jr., M.S., L.T. Ely, Ph.D., G.G. Wanless, B.Sc. and John Bahner, Jr., Ph.D. Linden, N.J.

API 06200

The foregoing are extracted from a publication entitled "Symposium on a Cancer Control Program for High-Boiling Catalytically Cracked Oils" reprinted with additions, from the American Medical Association Archives of Industrial Hygiene and Occupational Medicine, October 1951, Vol. 4, pp. 297-345.

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September 13, 1956

Doctors:	L. E. Curtis	K. R. Fourcher	J. W. Osborn
	V. C. Baird*	T. M. Frank	R. C. Page
	F. D. Gassaway	C. H. Hine	R. J. Potts
	W. O. Armstrong	W. F. Kahle	B. B. Reeve
	L. Blaney	T. J. Kelly	J. C. Ruddock
	F. F. Boys	E. K. Linder	George M. Saunders
	M. Clinton	E. P. Luongo	R. F. Schneider
	J. W. Crookshank	C. R. Miller	L. J. Wade
	Kieffer Davis	M. N. Newquist	E. R. Ware

Dear Doctors:

At the recent Gordon Research Conference on Toxicology, I was approached by Dean F. Davies, Ph.D., M.D., Administrator for Lung Cancer Research of the American Cancer Society, in order to determine whether industry might be interested in participating in a research project which the American Cancer Society is undertaking. In brief, the American Cancer Society is interested in determining whether screening lung X-rays offer any hope in prolonging the survival of lung cancer cases. In an attempt to do this, they wish to gather records of as many cases of lung cancer as possible, in which certain criteria were met. The criteria which they wish to meet are as follows:

1) Only records of patients who have been treated or discovered at least three years ago are wanted.

2) A follow-up on the survival time of each case will be necessary.

3) Only cases in which there have been normal X-rays within a year of the <u>first</u> appearance of a radiologic shadow are of interest.

4) A statement of the kind of therapy, if any, should be available. The date of surgery, if any, should be noted.

5) The age and sex of the patient must be known.

6) Only cases which have been verified by cytologic or pathologic diagnosis either during life or at autopsy are of interest.

7) All X-ray films on each such case will be borrowed for a period long enough to have them duplicated. They will then be returned to the original source so that radiologists can study the copies at their leisure.

The American Cancer Society, in this study, has absolutely no interest in the etiologic or possible etiologic aspects of the lung cancer problem. That is, they are completely unconcerned as to whether lung cancer may be due to smoking, air pollution, occupational hazards or other intrinsic, or as yet not well understood factors. Their sole interest is in determing whether or not screening lung X-rays offer hope in doing something about the rather distressing problem of lung cancer.

I promised Dr. Davies that I would contact each of you to inquire whether any of you have cases which meet the criteria outlined above, and whether you would be interested in participating in this research program by submitting case histories and X-rays. If you do have cases which meet the seven criteria and would be interested in such participation, you may communicate directly with Dr. Davies, whose full address is as follows:

Dr. Dean F. Davies
Administrator for Lung Cancer Research
American Cancer Society, Inc.
521 West 57th Street
New York 19, New York

Dr. Davies has suggested that it is possible that arrangements could be made for a statistical clerk to assist you if you have a sufficient number of cases which meet these criteria. Understand that any participation on your part will be on an individual and entirely voluntary basis.

I would like to emphasize, however, that I believe a study of this nature will have extremely beneficial results in our understanding of the course and possible methods of control of this ever increasing disease.

Sincerely yours,

R. E. Eckardt.

R. E. ECKARDT, M D.

REE:ejh
cc: Dr. D. F. Davies
Mr. D. V. Stroop

*This information is being provided to you under the terms of the Toxicological Research Agreement.

ESSO RESEARCH AND ENGINEERING COMPANY

(FORMERLY STANDARD OIL DEVELOPMENT COMPANY)

P. O. BOX 121, LINDEN, N. J.

MEDICAL RESEARCH DIVISION

ROBERT E ECKARDT PH. D. M D
DIRECTOR

N V HENDRICKS CH E
HEAD INDUSTRIAL HYGIENIST

HORACE W GERARDE PH D M D
HEAD TOXICOLOGIST

BERTRAND E BENNISON, M D
CHIEF CLINICAL RESEARCH PHYSICIAN

September 14, 1956

Messrs: [illegible] Adams; [illegible]; [illegible]; [illegible]; [illegible]; A. C. [illegible]

Gentlemen:

Attached for your information are copies of the following:

> The Report of the Symposium on Potential Cancer Hazards from Chemical Additives and Contaminants to Foodstuffs. International Union Against Cancer. Rome, 8/[illegible]/56.

> "The Potential Role of Non-Nutritive Food Additives and Contaminants as Environmental Carcinogens." Paper presented by Dr. W. C. Hueper before the above Symposium.

We have examined both reports and found no statement referring specifically to the carcinogenicity of "paraffins used for coating milk containers" for man and experimental animals, as reported in THE NEW YORK TIMES article of [illegible]/56.

We call your attention, particularly to Item 4, page [illegible] of the Symposium report, which reads as follows:

> "Since various petroleum constituents including certain mineral oils and paraffin, have produced cancer in man and experimental animals, the presence of such chemicals in foods appears to be objectionable, particularly when such materials are subjected to too high temperatures."

and to Item 12, page 4 of Dr. Hueper's manuscript which lists "wrapping and coating materials (paraffin, waxes, resins, plastics)" as materials which contaminate foods, apart from any considerations of carcinogenicity.

These reports should not be treated as publications since they have not yet appeared in the published literature, but rather were supplied to us by Dr. Hueper on the assumption that they would be used with appropriate discretion.

Very truly yours,

R. E. [redacted], M. D.

LCM:e,jh
Attach.
cc: (w/attach.) - Dr. L. K. Curtis
Mr. D. V. Strong

ESSO RESEARCH AND ENGINEERING COMPANY

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HORACE W. GERARDE, PH.D., M.D.
HEAD TOXICOLOGIST

BERTRAND E. BENNISON, M.D.
CHIEF CLINICAL RESEARCH PHYSICIAN

September 18, 1956

Messrs: E. W. Adams — R. C. Mithoff
C. H. Hine — M. N. Newquist
E. K. Linder — A. C. Pabst

Gentlemen:

This is to confirm that the next meeting of the Subcommittee on Carcinogenicity will be held in Chicago, Saturday, October 20, 1956. The place will be my room at the Drake Hotel. Unfortunately, I was unable to obtain a suite at the Hilton and so at the suggestion of Dr. Kehoe, I have managed to get a suite at the Drake. Incidently, for those who might not realize it, the Safety Council is meeting in Chicago at this time. For this reason, hotel reservations are scarce for October 20.

If any of you arrive late Friday night, October 19, it is probable that I will have an extra bed in my suite and I would be very happy to have you join me if you are unable to get a reservation. In fact, if necessary, we could probably have a couple of cots put up in the living room, if hotel reservations are that tight.

I am attaching to this letter, a copy of a proposal that was recently made to another company for studies on microcrystalline wax. These studies were really for the purpose of determining the chronic toxicity, in accordance with the recommendations of the FDA, and do not include tests of carcinogenicity. Even so, however, the total possible expenses of these studies comes to about $48,000.

The proposal which is under consideration by the FDA for testing of carcinogenicity, includes 300 rats and 800 mice (2 strains) on feeding experiments for 2 years, plus 400 rats and 400 mice (2 strains) on injection studies, plus 20 dogs on feeding studies for a total of 7 years. At the recent Gordon Research Conference on Toxicology, Dr. Fitzhugh of the FDA suggested that we might consider a compromise on the feeding studies, which would consist of 150 rats for 2 years with an additional 100 controls and 300 mice for 2 years with an additional 200 controls. This would then total some 750 rodents. They might consider 8 dogs with 4 controls for 5-7 years for cancer. In addition, they would probably require the 400 rats and 400 mice on injection studies. It is easy to see that if these recommendations are to be followed, the costs of such studies would be far in excess of the amount suggested in the attached.

I am simply enclosing this attachment in order to give each of you some understanding of the magnitude of the problem we are facing in planning to do carcinogenic studies on waxes. The preliminary discussions which we had with Dr. Kehoe and Dr. Horton in Chicago, suggested a theoretical approach to this problem, which, from a scientific standpoint, might be entirely satisfactory. As yet, I did not obtain any approval for use on milk cartons, at least from the carcinogenic standpoint. I sincerely hope that Dr. Kehoe, when he makes his proposal in Chicago, will indicate the practical facts of life, namely, those dealing with the FDA into consideration of making his proposal. All of us who have had dealings with the FDA in the past have found, at one time or another, that theoretical considerations, although carrying weight with the FDA, must, at times, be supplemented by rather practical considerations of what will be acceptable to the FDA.

It is my desire that Dr. Kehoe will have for us, a write-up of his proposal at least a week in advance of the October 20th meeting of the Subcommittee, in order that each of us may carefully review it prior to the meeting.

Sincerely yours,

R. E. Eckardt

R. E. ECKARDT, M. D.

REE:cjh
Attach. - PROPOSAL FOR STUDIES ON MICROCRYSTALLINE WAX. pp. 1-3.
cc: Dr. L. E. Curtis (w/o attach.)
Mr. A. W. Horton (w/attach.)
Dr. R. A. Kehoe (w/attach.)
Mr. D. V. Stroop (wo/attach.)

Dictated, but not read

Esso Standard Oil Company

15 WEST FIFTY-FIRST STREET · NEW YORK 19, N. Y.

R. L. MAY
VICE-PRESIDENT

September 20, 1956

Mr. Frank M. Porter, President
American Petroleum Institute
50 West 50th Street
New York 20, New York

Dear Mr. Porter:

Well over half of the wax produced by the United States petroleum industry goes into packaging of food. Throughout its long history in this service, the suitability of wax has come to be generally recognized. This acceptance has been due in a large part to the general level of purity voluntarily developed and maintained by the refiners producing it. Esso Standard feels this quality should continue on a voluntary basis in contrast to regulation. Recent events may foreshadow questioning of this quality.

You have no doubt had some knowledge of the symposium held in Rome August 10-15 by the International Union Against Cancer. While newspaper and magazine accounts may have lacked accuracy, they have focused new attention on paraffin wax as the petroleum product most intimately connected with foodstuffs. The symposium report begins --

> "The participants unanimously recognize the urgent necessity for international collaboration for the protection of mankind against exogenous (external) carcinogenic factors, and notably against carcinogenic substances that may be present in food. This collaboration should tend to encourage, help, coordinate and, if possible, support research so as to achieve practical results in the shortest possible time. Thus, the necessary scientific basis could be established for industries to plan their production and for governments of different countries to legislate for the effective protection of the health of consumers."

Having thus pointed up the problem and suggested avenues of enforcement, the Symposium Report, under (C) Food Contaminants, 4. reads --

> "Since various petroleum constituents including certain mineral oils and paraffin have produced cancer in man and experimental animals, the presence of such chemicals in foods appears to be objectionable, particularly when such materials are subjected to too high temperatures."

The report represents the combined comment of forty-two cancer experts from twenty-one countries.

In considering the future of petroleum wax in food packaging, we must face the fact that the symposium report links it directly with one of our most dire afflictions. Despite medical advances in diagnosis and control, cancer is still one of mankind's most dreaded words. At the same time, there is little biological or clinical data to substantiate the current industry level of paraffin or microcrystalline wax quality. While we may feel the association with cancer is ill-advised, we are hard put to prove it.

While the U.S. Pharmacopoeia acid test for paraffin has existed for several years and has been accepted by American Standards and the ASTM, its significance as a quality index is not unassailable. It is not suitable with microcrystalline wax and is not so used. It has yet to be linked biologically with food package use and few refiners warrant that their paraffin always passes it. In fact, the industry may be vulnerable in that, lacking a better index, we have failed to generally reach its level of purity especially for foodstuffs.

My company has done some medical research on paraffin and its potential cancer promoting tendency. We made limited use of these data in replies to questions on the Rome allegations. It may point to oil content of wax as an important characteristic. But it is not complete and must be extended to include microcrystalline waxes.

Up to this time there has been little if any cooperative effort between food container manufacturers and wax producers. This is especially true of the biological phases of wax quality. In general, the package maker tossed his questions on wax purity to the refiner or wax jobber and accepted the quality assurances he received. He used them to his own best advantage and enjoyed the general axiom that "everyone knows paraffin is okay for foods." This condition may not long endure.

There are four main trade associations in the food container field concerned over the symposium report and the general problem of acceptable petroleum wax quality. They are:

Liquid Tight Container Association
Paper Cup and Nested Containers Association
Waxed Paper Institute, Inc.
National Association of Sanitary Milk Bottle Closure Manufacturers, Inc.

I believe Mr. Humphries of API has already had correspondence with the paper cup group. I am not sure that the sanitary drinking straw manufacturers are represented but they should be interested as should Ex-Cell-O Machine which rents units for making paper milk containers. The position of all these and the petroleum industry would be so much stronger if there was a proven quality level for waxes used in food containers.

Historically, I do not know which API group has carried the responsibility of paraffin wax. It seems to involve both refining and marketing. But wherever the assignment falls, we must face the fact that we lack any proven level of

purity for this important public use. Without it both we and the container makers are vulnerable to questions arising from the Rome meeting or others similar to it.

The symposium report includes one general conclusion presented at the end. It reads --

> "The Conference recommends that, as a basis for active cancer prevention, the proper authorities of various countries promulgate and enact adequate rules and regulations prohibiting the addition to food of substances having potential carcinogenicity."

We should be prepared to establish petroleum waxes intended for food as suitable under such regulation. As a member of the Marketing Division I continue interested in the consideration given this problem.

Very truly yours,

R L Ray

RLR:FC

9-21-56

Date September 13, 1956

To Mr. D. V. Stroop Received 9-21-56 DVS

From: DR. R. E. ECKARDT

Possibly you might wish to distribute this to the members of the MAC.

R.E.E.

R. E. E. cjh

REE:cjn
Attach.

Copy From D. V. Stroop For Information Of Members Of MEDICAL ADVISORY COMMITTEE & TECHNICAL ADVISORS 9/21/56

DUSTON F

MINUTES OF THE MEETING OF THE SUBCOMMITTEE ON CARCINOGENICITY
Held in the Conrad Hilton Hotel in Chicago
Thursday, September 6, 1956

On Thursday, September 6, 1956, the Subcommittee on Carcinogenicity met at the Conrad Hilton Hotel in Chicago. In attendance were the following:

R. E. Eckardt, M. D. - Chairman
C. H. Hine, M. D.
P. Halley - (representing E. W. Adams)
E. K. Limper, M. D.
R. C. Mithoff
M. N. Newquist, M. D.

Representing the Kettering Laboratory were:

J. J. Phair, M. D.
R. A. Kehoe, M. D.
A. W. Horton

Two guests, namely, T. M. Frank, M. D. and Lamson Blaney, M. D., attended the meeting.

The Kettering Laboratory representatives joined the meeting at approximately 10:00 A. M

After approval of the report of the Subcommittee to the Medical Advisory Committee at its April meeting, the meeting was turned over to Dr. Horton. Dr. Horton reviewed the slides which he intends to use for his talk in Atlantic City before the American Chemic Society. The manuscript for this talk has already been approved by the MAC and the Medical and Health Committee of the Board.

Dr. Horton then submitted to the group the abstract entitled, "Relationships Between the Composition and Carcinogenic Properties of Various Refinery Streams." At the reque of the American Chemical Society, this abstract was prepared for distribution to report at the ACS meeting. The Subcommittee had certain suggestions concerning the abstract and these will be incorporated in subsequent revisions of the abstract by the Kettering Laboratory.

Dr. Horton next presented a table of his more recent data which is developing scientific support for his equation $P_{mc} = M_a(C_1 + C_2 + C_3)$. It will be remembered that this equatic has been qualitatively formulated by the Kettering Laboratory to express the carcinogenicity of any complex mixture.

In the formula, M_a represents a multiplication factor which is determined by the accelerators. C_1, C_2 and C_3 represent contributions from the carcinogens in the mixtures it being supposed that there are at least three classes of carcinogens. In the table submitted by Dr. Horton, the experiments with API NUMBER 89, suggest that at a 100 mg. dosage the P_{mc} is 0.3. This mixture was then chromatographed and separated into saturates through alkyl naphthalenes and total polycyclics. Biological testing of the

of the polycyclics yielded a P_{mc} of 0.04. This data suggested that M_a in the above formula is somewhere between 7 and 8. The polycyclics would represent the $C_1 + C_2 + C_3$ in the above equation. Next, Dr. Horton treated API NUMBER 89 with maleic anhydride. It will be remembered that such treatment yields an adduct with anthracene-type compound. It is not believed that two or three ring maleic anhydride adducts are carcinogenic, but it is believed that the 4+ rings, which react with maleic anhydride, contain the carcinogens of a benzanthracene type. Biological testing of this 4+ ring material yielded a P_{mc} of 0.012. Thus, this type of compound contributes about 1/3 of the carcinogenicity of the polycyclics. Conceivably, this figure, 0.012, might be taken to represent C_1 in the above equation. $C_2 + C_3$ would represent the difference between 0.04 and 0.012, and would represent the contribution of other classes of carcinogens, such as cyclopentano phenanthrene.

In the case of the API NUMBER 65, the P_{mc} of 0.11X is probably all contributed to by the polycyclics and of these, approximately 1/4 are by the benzanthracene-type compounds. Thus, a thermal tar has little, if any, accelerators and M_a in the above equation would be one. The rest of the data in the table is incomplete at the present time, but is being evaluated by Dr. Horton in the above fashion, in an attempt to determine the contribution of each of these constituents, to the over-all carcinogenicity of the mixture. It is Dr. Horton's hope that this work will permit a chemical quantitative estimation of the carcinogenicity of complex mixtures.

Following Dr. Horton's presentation the chair was turned over to Dr. Fhair, who described what they hoped to accomplish with the epidemiological data thus far collected. It is his hope that among the 2,000+ cases thus far collected, they will at least be able to provide us with some figures on relative frequencies of different types of cancer within the petroleum industry. Dr. Fhair emphasized that he did not believe the epidemiological work had been a total failure, since he believes that secondary benefits, such as making medical directors think in terms of disease reporting and the collecting of disease statistics, had been accomplished by the abortive attempt to collect epidemiological data. It is possible that this attempt within the petroleum industry was just 10 years ahead of its time.

Subsequent to these discussions, the Subcommittee discussed the present situation concerning milk carton wax which had been precipitated by a recent article in THE NEW YORK TIMES. It was the general consensus that the following represented a fair estimate of what we should do at the present time.

1) No public statement should be made by the API at the present time.
2) The Chairman of the Subcommittee should distribute to all members of the MAC, a memorandum summarizing presently available information on the carcinogenicity of milk carton wax. Such distribution will be dependent upon his being able to obtain clearance from his management.

3) The chairman of the Subcommittee, after receiving Dr. Hueper's Rome manuscript and the resolutions of the Rome symposium, should write a letter to the Surgeon-General, with a carbon copy to Dr. Hueper, asking for clarification of the statements which appeared in THE NEW YORK TIMES. This should be done on API letterhead and the tenor of the letter should be dependent upon the text of Dr. Hueper's manuscript and the resolutions adopted in Rome. The letter will be circulated to the Subcommittee prior to being forwarded to the Surgeon-General.

4) It was the general consensus that insufficient data is presently available to permit adequate evaluation of the carcinogenicity of dairy waxes. The Kettering Laboratory was asked whether they would be interested in conducting the research necessary to clarify and establish the status of dairy waxes in regard to their carcinogenicity. The Kettering Laboratory expressed interest in this research program and promised to prepare definite proposals for such research with budget estimates as to the cost for a subsequent meeting of the Subcommittee, to be held in Chicago on October 20, 1956. Tentatively, the place of this meeting will be at the Drake Hotel, providing the chairman of the Subcommittee can obtain adequate accommodations for a meeting room for the Subcommittee on that date. At this time, the Kettering Laboratory will have specific proposals with, more or less, specific budgetary estimates for the research necessary to clarify this situation. In the meantime, the chairman of the Subcommittee will provide to Dr. Kehoe, a copy of the memorandum which is to be distributed under item #2 above.

Dr. Kehoe was reminded, just before the meeting adjourned, that there would be a meeting of the Subcommittee about the time of the IMA meeting in April and at this time, he would be expected to present budgetary figures for the year July 1, 1958 to June 30, 195

R. B. ECKARDT, M. D./cjh
Chairman, Subcommittee on Carcinogenicity
September 10, 1956

Attach. - ABSTRACT RELATIONSHIPS BETWEEN THE COMPOSITION AND CARCINOGENIC PROPERTIES OF VARIOUS REFINERY STREAMS.
A. Wesley Horton, et al.

cc - D. V. Stroop
J. A. Kehoe

REE
LCM
DFB
JMG

ABSTRACT

RELATIONSHIPS BETWEEN THE COMPOSITION AND CARCINOGENIC PROPERTIES OF VARIOUS REFINERY STREAMS

by

A. Wesley Horton, Russell Tye and Mary Jane Graf

Kettering Laboratory, University of Cincinnati

Various high-boiling mixtures derived from fossil fuels have the capacity to produce tumors when applied repeatedly upon the skin of certain experimental animals for long periods of time. An extensive program of testing of such materials encountered in petroleum refining operations has been undertaken (at the request of this industry).

A technique of analysis for benzopyrenes in such oils has been developed. Application of this method to a wide variety of oils has shown that knowledge of the concentration of this carcinogen alone is not sufficient for reliable estimation of the carcinogenic potency of a particular oil.

Further research indicated that very important contributions to the potencies of certain oils are made by classes of hydrocarbons which are completely non-carcinogenic when separated and tested by themselves. The exact mechanism of their action in mixtures with polycyclics such as benzopyrene is unknown, but their effect is to shorten the latent period before the development of tumors.

Work is also in progress to identify the important carcinogens of certain active oils which are essentially free of benzopyrenes. The techniques of chromatography, distillation, solvent extraction, and the formation of Diels-Alder adducts have been used to produce relatively simple mixtures. Some biological testing of these fractions has been made and additional testing is under way. The approximate composition of these fractions has been determined by absorption and mass spectrometry. A strong likelihood is indicated that the unknown carcinogens are 4-ringed hydrocarbons, but different in structure from the classical benzanthracenes and chrysenes.

API NUMBER	DESCRIPTION	100 mg. P_{MC}	CHROMATOGRAPHIC FRACTIONATION: Saturates thru Alkyl-naphthalenes (%)	CHROMATOGRAPHIC FRACTIONATION: Poly-cyclics (%)	CONTRIBUTION OF POLY-CYCLICS TO P_{MC}	FRACTION EXTRACTED BY MALEIC ANHYDRIDE: % Yields 2-3 rings	FRACTION EXTRACTED BY MALEIC ANHYDRIDE: % Yields 4+ rings	FRACTION EXTRACTED BY MALEIC ANHYDRIDE: Contribution of 4+ Ring Fraction to P_{MC}
89	FCC Sidestream	0.3						
89-1						1.64		-
89-2							0.2	0.012
89-3				19	0.04			
89-4			81%					
65	Thermal tar	0.11X						
65-1				60	0.12			
65-2						(0.9)	1.1	0.03
120	Straight run distillate	0.07 (50 mg.)						
120-1						(1.2)	0.4	<0.01
120-2				33	inc.			
33	Coker gas oil	0.4						
33-3						(2.1)	2.2	inc.
33-4				33	inc.			
105	Straight run distillate	0.11						
115	Straight run distillate	0.03						

A direct test of this fraction has produced 1 benign papilloma in 28 weeks.

Date September 13, 1956

To Mr. D. V. Stroop: RECEIVED 9-21-56 DVS

From: DR. R. E. ECKARDT

Possibly you might wish to distribute this to the members of the MAC.

R.E.E.

R. E. E. cjh

REE:cjh
Attach.

Copy From D. V. Stroop For Information of Members Of MEDICAL ADVISORY COMMITTEE & TECHNICAL ADVISORS 9 21 56

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Chairman, Subcommittee on Carcinogenicity
September 10, 1956

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cc - D. V. Stroop
R. A. Kehoe

=EE 4
LCM ✓
BEB ✓
DWG ✓

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by

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						2-3 rings	4+ rings	
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89-1						1.64		-
89-2							0.2	0.0
89-3				19	0.04			
89-4			81*					
65	Thermal tar	0.11X						
65-1				60	0.12			
65-2						(0.9)	1.1	0.03
120	Straight run distillate	0.07 (50 mg.)						
120-1						(1.2)	0.4	<0.01
120-2				33	inc.			
33	Coker gas oil	0.4						
33-3						(2.1)	2.2	inc.
33-4				33	inc.			
105	Straight run distillate	0.11						
115	Straight run distillate	0.03						

* A direct test of this fraction has produced 1 benign papilloma in 28 weeks.

Socony Mobil Oil Company, Inc.

150 EAST 42ND STREET NEW YORK 17, N Y

24 September 1956

To: API Subcommittee on Carcinogenicity
Messrs. R.J. Schmidt, M.D. - Chairman
E.W. Adams
C.H. Hine, M.D.
H.K. Linder, M.D.
R.C. Mithoff
H.H. Rovquist, M.D.

Subj: Paraffin Wax

I believe the API Subcommittee on Carcinogenicity will be interested in the attached letter which was sent by Dr. W.C. Hueper to Dr. Ernest Reid at Syracuse University concerning the statements made by the former at the recent Rome Conference.

In my opinion, Dr. Hueper has a very sensible approach and is in conformity with present thinking of the subcommittee -- namely, that comprehensive experiments on paraffins of different derivation and purity be made.

It is possible the subcommittee may like to cooperate with or approach Dr. Hueper regarding the initiation of such test work at the National Institute of Health because it seems Dr. Hueper is primed to do this work and may do it independently.

This copy of Dr. Hueper's letter was received "through channels" and I, therefore, believe it should be held more or less in confidence.

Very truly yours,

A. C. Pabst

ACP:jd
attachment
cc: Mr. D.V. Streep + att.

API 06225

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
Public Health Service
National Institutes of Health
Bethesda 14, Md.

September 5, 1956

Dr. Ernest Reed, Director
Microbiological & Biochemical Research Center
Syracuse University
Syracuse 10, New York

Dear Doctor Reed:

Under separate cover, I am sending you copies of my report given at the Rome Conference and of the official report of the Symposium. It goes without saying that I did not claim that paraffins used for food containers have been shown to cause cancers in man. As far as I am aware, there do not exist any publications on this particular point. However, recent years have brought to my office several inquiries as to the safety of such containers from a cancer viewpoint. With the more recently acquired knowledge on carcinogenic fractions obtained from petroleum and related products, and considering the fact that paraffins in other countries are also produced from lignite oils and shale oils, which as a rule are more carcinogenic than the corresponding petroleum oils, it may be wise in my opinion to perform some comprehensive experiments on paraffins of different derivation and purity, so as to be certain that paraffins which may not be entirely safe are not used for the preparation of food containers. As the result of such investigations, I would expect that the interested industries will be able to adopt and develop standards insuring the safety of paraffins employed for such purposes.

Perhaps it may also be advisable to ascertain during such experiments whether paraffin flakes from the wall of food containers, such as milk cartons, may decompose into the milk, and whether foodstuffs containing fats may extract some constituents of the paraffin used for impregnating food containers and wrapping material, especially if the food containers are filled with hot liquids.

Sincerely yours,

/s/ W. C. Hueper
W. C. Hueper, M. D., Chief
Environmental Cancer Section
National Cancer Institute

September 10, 1956
Forwarded:

Ernest Reed, Director
Microbiological & Biochemical
Research Center
Syracuse University
Syracuse 10, New York

API 06226

ESSO RESEARCH AND ENGINEERING COMPANY

(FORMERLY STANDARD OIL DEVELOPMENT COMPANY)

P. O. Box 121, Linden, N. J

MEDICAL RESEARCH DIVISION

ROBERT E. ECKARDT, PH., M.D
DIRECTOR

N. V. HENDRICKS, CH E.
HEAD INDUSTRIAL HYGIENIST

HORACE W. GERARDE, PH.D., M.D
HEAD TOXICOLOGIST

BERTRAND E. BENNISON, M.D
CHIEF CLINICAL RESEARCH PHYSICIAN

September 24, 1956

Mr. D. V. Stroop
American Petroleum Institute
50 West 50th Street
New York 20, New York

Dear Mr. Stroop:

I am sending you minutes of the last meeting of the Committee on the Carcinogenic Action of Mineral Oils of the British Medical Research Council.

I believe it would be useful to have these duplicated and forwarded to the members of the Medical Advisory Committee for their information. Dr. Horton should also be included on the distribution list.

Sincerely yours,

R. E. Eckardt

R. E. ECKARDT, M. D.

REE:cjh
Attach. - MEDICAL RESEARCH COUNCIL. Committee on the Carcinogenic Action of Mineral Oils. MRC.56/727 CAMO. Min.20 pp. 1-3.

- MEDICAL RESEARCH COUNCIL. Committee on the Carcinogenic Action of Mineral Oils. Data Tables of Mineral Oil Fractions. MRC.56/731 CAMO.56/8 pp. 1-2.

Copy From D. V. Stroop For Information Of Members Of Medical Advisory Committee 9/26/56

MEDICAL RESEARCH COUNCIL

"Unclassified"
For Specified Circulation
(Members of Committee)

MRC.56/731
CAMO.56/8

FOR INFORMATION

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

DATA TABLES OF MINERAL OIL FRACTIONS

Amendments and additions to section 2, of MRC.56/542, CAMO.56/3, submitted by Dr.D.L.Woodhouse and Dr.Philip J.King, (Min.3(d) of 20th meeting).

Page K.1 add note to Fraction K.4.4 (390-400°C)

All used up. Fraction is no longer available.

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add K_4S_9A, K_4S_9P

Page K.4c Footnote ϕ delete A & b, add a & b

Page K.5 delete whole page and add page K.5 attached.

Page K.6 add new fractions

KX_1	KX_2	KX_3	KX_4	KX_5
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KX_6	KX_7	KX_8
(375-380°C)	(380-385°C)	(385-390°C)

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Page L.3 add (to all aromatics) 7

add L_4SP 7

add L_4S_9A, L_4S_9P

Page O.2 delete "Steadman Still" add "Stedman Still"

Page O.3 add O_4SP. O_4S_9A, O_4S_9P

NOTE:- Fractions L_4SP and O_4SP correspond to the K_4SP fraction, that is to say that they were prepared by making a blend from equal volumes of the fractions L_4S_1P ------ L_4S_8P (and O_4S_1P ------ O_4S_8P). They were made in order to test the biological activity of the 'non-aromatic' portion of the oil after acetone treatment.

Further Separation of Atomatic Fractions

$K_4S6_{Ab}r$, $K_4S7_{Ab}r$, $K_4S7_{Ab}r$, $K_4S8_{Ab}r$, $K_4S8_{Ab}r$

↓

Each of the above fractions chromatographed on Alumina, yielding in each case

Petrol.Ether Eluate — Benzene Eluate — Methanol Eluate

↓

Corresponding Eluates from all fractions combined to give bulked samples

↓

'Pet.Ether' — 'Benzene' — 'Methanol'

$K_4S6_{-8}AriV$ — $K_4S6_{-8}AriiV$ — $K_4S6_{-8}AriiiV$

↓

Each bulked sample distilled on the 'Spinning Band Column', and equal volume fractions taken.

$K_4S6_{-8}AriV$	$K_4S6_{-8}AriiX$	$K_4S6_{-8}AriiiX$
$K_4S6_{-8}AriW$	$K_4S6_{-8}AriiY$	$K_4S6_{-8}AriiiY$
$K_4S6_{-8}AriX$	$K_4S6_{-8}AriiZ$ (Residue)	$K_4S6_{-8}AriiiZ$ (Residue)
$K_4S6_{-8}AriY$		
$K_4S6_{-8}AriZ$ (Residue)		

MEDICAL RESEARCH COUNCIL

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(Members of Committee)

MRC 56/727
CAMO. 56/20

FOR INFORMATION

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

Minutes of the twentieth meeting held at 38 Old Queen Street, London, S.W.1, on Tuesday, 3rd July, 1956 at 2.15 p.m.

MEDICAL RESEARCH DIVISION
REE
BEB
NVH
HWG
FWC
ARJ
JDY
LCM
CH
MK
SEP 17 1956

Present: Professor T. Ferguson (Chairman), Col. S.J.M. Auld, Dr. J.W. Cook, Professor H.N. Green, Dr. I. Hieger, Dr. J.O. Irwin, Professor F. Morton, Professor R.D. Passey, Professor J.R. Squire, Dr. D.L. Woodhouse, with Dr. R.C. Norton (Secretary).

Attended: Dr. Philip J. King, and Dr. W. Carruthers.

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The Minutes of the nineteenth meeting were approved and signed.

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New Rapid Screening Tests for Possible Carcinogens

Professor Squire said that he had written to Dr. W.E. Smith (Minute 158 (ii)), but that he had not yet received a reply.

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Dr. Carruthers drew attention to the high percentage of sulphur compounds present in the oil, most of those isolated being dibenzthiophen derivatives. He was, however, disappointed that it had not been possible to isolate many of them in a pure state and then only in small amount.

Dr. Cook drew attention to the publication by Dr. Carruthers of papers describing the isolation of certain of the compounds:
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"The Constituents of High-boiling Petroleum Distillates. Part III. Anthracene Homologues in a Kuwait Oil". W. Carruthers, J. Chem. Soc., 1956, 603.
"Triterpenoids. Part XXI. A Triterpenoid Lactone from Petroleum". D.H.R. Barton, W. Carruthers and K.H. Overton, J.Chem.Soc.,1956, 788.

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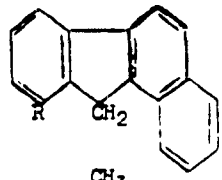
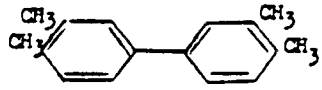
Formula (I) should read as follows:



and formula (II) as follows:



He also pointed out that in the table on page 2 of his report (Chromatographic Separation of Picrate-forming Fractions from Kuwait Oil), the figure in the last column opposite K4S6b should be 17.5 and not 175.

(b) by Professor F. Morton (CAMO 56/7)

Referring to table 1 in his report, Professor Morton said that the fractionation of eluates supplied by Dr. Carruthers had been disappointing. The work had had to be undertaken hurriedly and, at that time, they were inexperienced in the necessary technique. The first few distillations had therefore been inefficient. The ultimate picrate-forming fractions, although the distillation curve had been good, were also disappointing in that there had been no marked separation into chemical types. He thought that a more efficient method of fractionation would have to be found than any which had been used to date. For the distillation of the highest fractions it might be necessary to purchase a molecular still. He thought, however, that this molecular still would not be needed until the large scale picrate separations had been completed.

He pointed out that the tables from page 14 onwards in his report brought Dr. Woodhouse's data tables (L3 and 03) up to date.

Professor Morton referred to the approaches which he had been making to the Dyestuffs Division of Imperial Chemical Industries at Blackley, to see whether they could undertake in their pilot plant the large scale separation of compounds using picric acid. The Committee approved of these approaches, and agreed that Professor Morton should discuss details of the whole work with I.C.I. representatives on a confidential basis.

The Committee also noted that Professor Morton intended to publish the completed analytical data on the K, L and O oils.

(c) by Dr. D.L. Woodhouse - Progress Report No. XVI (May 1956), (MRC.56/542, CAMO.56/3)

Dr. Irwin said that a point of great significance emerging from this report was the failure to recover the original carcinogenic activity in the sub-fractions tested during 1955. The sub-fraction results showed that at least 50 per cent of the activity originally present seemed to have disappeared. Possible causes for this loss of activity were discussed. These included consideration of whether there had been a loss of promoters, e.g., by crystallisation with picric acid. It was suggested that it might, as a check, be disirable to re-blend the separated materials and then test the resulting mixtures to see if the original level of carcinogenic activity had been recovered. Professor Morton thought it unlikely that there had been any significant loss or chemical change during the processes of fractionation.

Professor Passey suggested that the carcinogens might in fact still be present, but that their concentration had fallen below the level of biological activity. He referred to the work on tobacco, and pointed out that, although benzpyrene had been isolated from all the tobacco tars, no tumours had yet been produced in this country with any of these tars.

Professor Squire mentioned the possibility that there might be some arithmetical fallacy connected with the dilution of the fractions with liquid paraffin. This, however, was generally felt to be unlikely.

The Committee agreed that the reasons for this loss of activity required the most careful consideration.

Referring to other aspects of Dr. Woodhouse's report, Dr. Irwin said that the results with mice were similar to those obtained in previous years and had not shown any discrimination between fractions. He therefore queried the desirability of continuing these experiments in future years. Dr. Cook said that even if there were to be no more experiments with mice using the K fractions, he felt that the experiments should, nevertheless, be continued with those from the L and O oils. Dr. Woodhouse said that he would like to continue the mice experiments with all the oils, and the Committee agreed in principle that this should be done.

(d) Data tables in mineral oil fractions, submitted by Dr. D.L. Woodhouse - (Section 2 of MRC.56/542, CAMO.56/3)

Dr. King pointed out some errors in these tables and he agreed, in conjunction with Dr. Woodhouse, to submit amendments for circulation.

(e) Experiments with mice at Leeds, (MRC.56/549, CAMO.56/6)

Dr. Irwin said that the results obtained agreed well with those obtained at Birmingham.

4. Future plans

(a) Chemical - It was pointed out that activities would, for the time being, have to be to some extent limited due to the moves of Professor Morton and Dr. Carruthers to Manchester and Exeter respectively.

It was thought desirable that the separations, which had so far been performed by Dr. Carruthers on a small scale, should now be repeated on a larger scale and that this picrate separation should then be followed by chromatography of the p and r fractions, instead of by their fractional distillation.

(b) Biological - (i) Birmingham - It was suggested that the work at Birmingham should continue to be on the K series. The Committee agreed that Professor Squire, Professor Morton and Dr. Woodhouse should meet early in September to discuss detailed arrangements.

(ii) Leeds - It was agreed that the next series of tests at Leeds should be on the K_4S_9 series and the equivalent L and O fractions, making 6 fractions in all. In addition to these Dr. Carruthers agreed to provide a further 12 fractions from the picrate series.

Professor Green suggested that now his facilities were being increased, he might undertake a series of pilot experiments, in addition to the large scale work, in the hope of picking out quickly further fractions of high carcinogenic activity. Dr. Irwin said that he would look into the possibility of evolving a more flexible design for testing about 5 fractions, using about 10 rabbits for each.

5. Next meeting

It was agreed that the next meeting should be held about the first week in December.

MEDICAL RESEARCH COUNCIL

"Unclassified"
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FOR INFORMATION

MRC.56/731
CAMO.56/3

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

DATA TABLES OF MINERAL OIL FRACTIONS

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add K_4S_9A, K_4S_9P

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Further Separation of Atomatic Fractions

K_4S_6Ar, K_4S_7Ar, K_4S_7Ar, K_4S_8Ar, K_4S_8Ar

↓

Each of the above fractions chromatographed on Alumina, yielding in each case

Petrol.Ether Eluate	Benzene Eluate	Methanol Eluate
↓	↓	↓
Corresponding Eluates from all fractions combined to give bulked samples		
↓	↓	↓
'Pet.Ether'	'Benzene'	'Methanol'
$K_4S_{6-8}Ari^{\ddagger}$	$K_4S_{6-8}Arii^{\ddagger}$	$K_4S_{6-8}Ariii^{\ddagger}$
↓	↓	↓
Each bulked sample distilled on the 'Spinning Band Column', and equal volume fractions taken		
↓	↓	↓
$K_4S_{6-8}AriV^{\ddagger}$	$K_4S_{6-8}AriiX^{\ddagger}$	$K_4S_{6-8}AriiiX^{\ddagger}$
$K_4S_{6-8}AriW^{\ddagger}$	$K_4S_{6-8}AriiY^{\ddagger}$	$K_4S_{6-8}AriiiY^{\ddagger}$
$K_4S_{6-8}AriX^{\ddagger}$	$K_4S_{6-8}AriiZ^{\ddagger}$ (Residue)	$K_4S_{6-8}AriiiZ^{\ddagger}$ (Residue)
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MEDICAL RESEARCH COUNCIL

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MRC.56/727
CAMO. 56/8C

FOR INFORMATION

COMMITTEE ON THE CARCINOGENIC ACTION OF MINERAL OILS

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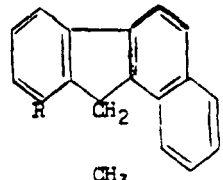

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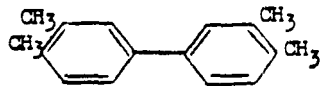

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Copy From D. V. Stroop For Information Of Members Of Medical Advisory Committee 10 2/56

DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
FOOD AND DRUG ADMINISTRATION
WASHINGTON 25, D. C.

September 20, 1956

Mr. Homer N. Calver
Secretary, Public Health
Committee of the
Paper Cup and Container Institute
Two-Fifty Park Avenue
New York 17, New York

Dear Mr. Calver:

Reference is made to your letter of September 12, 1956 requesting my comments on news reports of the recent Rome meeting of the International Union Against Cancer. You particularly ask if there is evidence that carcinogens are involved in food additives used in this country as some of the news items apparently imply.

We have seen some news items and we have had a number of inquiries about these reports. We agree that some of the chemicals mentioned are unsuitable for food use, although we are not certain that the evidence as to carcinogenicity is complete. However, we are not aware of evidence that any of the food wrappers or containers, chemical additives, or food colors in use in this country are, in the manner used, carcinogenic. If such evidence were available, of course, we would take appropriate action against such use under the Food, Drug, and Cosmetic Act.

We do believe that a potential public health problem does exist as a result of the production of vast numbers of new chemicals in recent years, many of which have been proposed for uses that would, either deliberately or inadvertently, add them to food products. Sufficient information regarding the chemistry and possible toxicity of many of these new chemicals is not available. In the absence of evidence of toxicity or harmfulness we would not be able under the present law to prevent the use of these new additives. It has been our view that provision should be made in the law to require that a chemical by appropriate tests be proved innocuous before it may be employed as a food additive. You may know that the Congress has had under consideration a number of proposed amendments to the Act which, in line with the report of the House Select Committee to Investigate the Use of Chemicals in Foods and Cosmetics (Report 2356, 82d Congress, June 30, 1952), would provide a legal basis and procedure for

Mr. Homer N. Calver

requiring proof of safety before additives could be used as ingredients of food products.

I am not sure just when a report of the Rome meeting will be available. Committees were appointed to prepare reports on the various matters, which will be circulated among participants before they are prepared for release. I am enclosing a copy of the paper which I submitted at this meeting. I think it indicates our views with respect to the problem of food additives.

I believe that some of the items in the newspaper reports that you mentioned were based upon Dr. Hueper's paper. You perhaps have made inquiry of him as to the information which he presented at these meetings.

Sincerely yours,

A. J. Lehman, M.D.
Chief, Division of Pharmacology
Bureau of Biological and Physical Sciences

Enclosure
Problems of Food
Safety

Presented in Rome, Italy,
at Meeting of Natl. Comm.
on the International Union
Against Cancer, Aug. 10-15, '56

Problems of Food Safety

by
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The safety of food has been of concern to man from the very earliest time. Trial and error must have played a large part in estimating hazards. No doubt fatal errors must have taught which foods could be eaten without harm. It is also safe to say that the problems of food safety in these early times dealt with acute poisonings. Food safety problems of modern civilization rarely concern those substances, natural or otherwise, that result in immediate harm. On the contrary, it is the insidious hazards of chronic toxicity that are associated with the problems of today.

The current problems of food safety fall into three general groups.

1. Food production. In this category is a long and lengthening series of chemical substances which include insecticides, fungicides, herbicides, plant growth regulators, insect repellents, defoliants, animal growth stimulants, crop protectants, and fumigants.

2. Food processing. In processing, foods may receive preservatives such as antioxidants and antimycotics, bleaches, colors, flavors, deodorizing treatment, humectants, drying agents, thickening agents, sequestering agents, sweetening agents, stabilizers, emulsifiers, neutralizers, acidifiers, anticaking and antifoaming agents. These and other agents may influence practically every property of natural food.

In addition one must also recognize the possibility of inadvertently introducing still other substances such as cleaning and sanitizing agents, leachings from the surface of processing equipment, and even lubricants.

An excellent example of the latter is the x-disease or hyperkeratosis of cattle caused by chlorinated naphthalenes getting into pelleted food via lubricants.

3. Food packaging. The finished food is commonly distributed in packaging materials which contain synthetic resins, plasticizers, stabilizers, pigments, and other components, all of which may contribute additives to the food.

Food Production and the Pharmacological Problems of Crop-Protecting Chemicals

There is no question about the necessity for pesticides in the production of food crops and even in some instances in the protection of the stored crops after harvest. By and large, these chemicals are poisonous and in their use there is apt to be a residue on the treated crop. Our major pharmacological problem, however, is not to determine whether or not the pesticide can cause death, but rather the appraisal of the hazards associated with the continued use of agricultural products treated with normal amounts of the pesticide.

The accepted experimental procedure for the evaluation of the safety of a pesticide is conducted on the basis of determining what effects may be produced by lifelong feeding studies in a short-lived animal. This procedure has been comprehensively described in an article entitled Procedures for the Appraisal of the Toxicity of Chemicals in Foods, Drugs. and Cosmetics prepared by members of the Division of Pharmacology of the Food and Drug Administration, and published in the Food Drug Cosmetic Law Journal for October 1955.

This procedure employs carefully selected animals kept in a uniform environment and whose only challenge is the pesticide under study. The normal order of affairs for the human are not homogeneity, uniform environment, or confrontation with but a single challenge. For this reason it would be desirable to supplement the accepted experimental procedure with groups of tests representing animals under stress. However, this is not entirely feasible because of the number of stress conditions that could be introduced and the number of animals involved in the tests would rapidly outstrip laboratory facilities.

A supplementary approach which we have employed consists of determining what proportion of an administered test dose that is absorbed, what proportion is destroyed or modified by metabolism, and what proportion is stored in the tissues. We also vary this technique by putting a stress on the dynamic equilibrium established by the experiment just mentioned. This consists of determining the level of intake at which the pesticide or its metabolite produces a deviation from the normal pattern of absorption, metabolism, and storage.

The problem of the carcinogenetic activity of pesticides also is carefully considered. Probably more is read into this aspect than is warranted. Nevertheless, pesticides with structural relationships to known carcinogens, and those that cause definite morphological alterations, are especially scrutinized. Pesticides that are stored like wise are considered possible carcinogens and investigated accordingly. Chronic exposure to the test substance becomes most important. A few of the many reference points which have been of considerable value in evaluating

carcinogenicity are (1) the disappearance point, i.e., the highest dosage level in a chronic feeding study at which tumor formation cannot be observed on histopathological examination; (2) the point at which mortality is affected by the test substance, i.e., the lowest dosage level which influences the mortality of the animals under test; and (3) the fifty per cent point, i.e., the dietary level of the pesticide at which approximately half of the animals show tumor formation. These reference points for DDT are: (1) disappearance point, 300 ppm dietary level; (2) effect on mortality, 500 ppm; and (3) the fifty per cent point, undetermined because the dosage level which is apparently necessary to produce this effect cannot be tolerated by the animals and death results before tumors are induced. DDT is not classed as a carcinogen because even at the dietary level at which tumor formation was reported there was only a minimal and late tendency in the formation of hepatic cell tumors. The tumors themselves were benign rather than malignant on histological examination. The relative case of rat liver tumor formation, the relative scarcity of human primary liver tumors, and the whole problem of translating animal data into human terms, must of course be considered. As of the present writing we feel that no pesticide used in agricultural practice in the United States has shown carcinogenetic tendencies in animals such that the residues ingested by man would be a danger. Only one (Aramite, a chlorinated miticide) has produced tumors with any facility, and a residue of 1 ppm is being allowed while more extensive studies of its carcinogenic potency are being conducted.

Food Processing and Intentional Chemical Additives

As already mentioned chemical additives are added as retainers, modifiers, and inhibitors of practically every property natural food may exhibit. In the United States we proceed on the premise that no citizen is expendable within the meaning of the Federal Food, Drug and Cosmetic Act. The pharmacological techniques and procedures which we follow to evaluate the safety of a food additive are essentially the same as those applied to pesticides. Those of us who are responsible for safeguarding the food supply do not indulge in the educated guess or the calculated risk. Decisions regarding the safety of a proposed food additive involve many considerations including carefully designed experimental studies and a critical analysis and interpretation of the data. A given usage is considered safe only if the maximum safe dose in long-term animal feeding studies is at least 100 times the amount that may occur in the total human diet. Among the reasons for such a high factor is the generally greater resistance of animals versus man to toxicants. All of the additives presently used in the United States which have been informally cleared with us have met this criterion. In general if a proposed additive meets the basic principles as defined by the Food Protection Committee of the National Research Council, its safety can be considered as being established to a reasonable degree.

The possible carcinogenicity of food additives has also been the subject of critical scrutiny. At this point it may be informative to sketch briefly the reasons why an effective preservative and antioxidant, thiourea, is not permitted in foods. If we apply the same reference

points mentioned in connection with DDT the following facts are revealed. The disappearance point at which the continuous ingestion of thiourea in the diet of rats does not cause tumor formation is about 50 parts per million. Mortality is affected at 1500-2000 ppm. The fifty per cent point, or the level of feeding at which approximately half of the exposed animals develop tumor, is between 100 and 500 ppm. These latter values approach the use level of thiourea as a direct additive to foods. Other uses which have been proposed are far in excess of the 100-500 ppm concentration. A concentration of 2 to 4% was proposed in a dip for oranges.

In this connection the use of thiourea as a component of household silver polishes may be questioned. This is permitted in the United States. Man is less sensitive to the acute effects of thiourea than to the chronic ones. Furthermore, both thiourea and the salt of the silver complex formed during the polishing procedure are water soluble. Thus, the occasional use of the polish followed by rinsing should not contribute a significant residue to food which comes in contact with the treated silver.

There has been much speculation and uninformed opinion regarding the safety of food colors. The only food colors we are concerned with are the 19 certifiable FD&C colors. None of these has yet shown evidence of carcinogenicity in man or in feeding tests with experimental animals. Some, particularly in the triphenylmethane class, have produced fibrosarcomas at the site of repeated subcutaneous injection in rats. This site, known to be a sensitive one, and only recently coming into common use in

carcinogenicity testing, is of course an abnormal one as far as food colors in the diet are concerned. However, it is a warning against the use of these colors in parenteral drugs, as has been proposed. To the extent that time and limited facilities permit, the Division of Pharmacology is engaged in more detailed animal toxicity studies with these colors than has heretofore been done.

Three colors, FD&C Orange No. 1, FD&C Orange No. 2, and FD&C Red No. 32, have been withdrawn for food and drug use in the United States. This action has not been based upon carcinogenicity but upon toxicological evidence which showed that these dyes could no longer be classed as harmless.

Substances which have been found to be carcinogenic in recent years and which, for that and other reasons, the Food and Drug Administration has not permitted in foods include dulcin, a synthetic sweetener, and selenium compounds as insecticides.

Emulsifiers presently used in foods in the United States are not carcinogenic by any of the criteria that have been applied. One property of emulsifiers, their surfactant effect, was the cause of some concern. However, the Food Protection Committee of the National Research Council has reviewed the literature on the subject and has stated that the surface acting effect of emulsifiers and wetting agents is no measure of their toxicity. This means that each compound must be individually investigated for toxicity with the surfactant effect only an icidental property which need not be considered in its safety evaluation. Based upon this a broader use of emulsifiers may be permissible in foods than heretofore.

The Food Protection Committee's statement also has a bearing on

the use of surfactants as detergents and sanitizers for food processing equipment. If harmlessness of these compounds can be established by the accepted procedures, contamination of food by equipment cleaned with these agents becomes less of a hazard than in our previous estimates. A problem which appears to be confused in the minds of some is the use of "polyphosphates" as detergents. In our opinion the linear phosphates, sodium hexametaphosphate for example, are harmless substances and present no hazard when employed for cleaning purposes. Until proven otherwise we are of the opinion that cyclic phosphates are deleterious substances and their use where food contamination may result would not be permitted.

Chelating agents are another class of compounds which may find greater application in the food field. Since a chelating agent chelates different metals in different degrees, this property must be taken into account in its final evaluation of safety. One of these, phytic acid and its calcium salt, is well on its way toward being established as acceptable for a number of food applications. The familiar ethylene diamine tetraacetic acid, EDTA, has not yet been established as safe for use in foods.

Flavoring agents have not received as much attention as other food additives. Some two years ago a survey was made to determine the approximate number of flavoring agents in more or less common use. A list of about 250 flavor chemicals was assembled and classified into five chemical groups. In order to proceed in some orderly fashion in the investigation of these aromatics, a primary list representing about 10 compounds for each of the classifications was prepared and several

candidates were chosen from each classification for study. Before these studies were undertaken our Division of Food undertook to characterize chemically each of the flavors, since a reasonable degree of purity has to be established before the flavor was considered acceptable for inclusion in our program. The five classifications are ethers, esters, phenols, aldehydes, and alcohols. The general plan has been to feed these compounds at levels of 1%, 0.25%, and 0.1% in the diet of weanling rats for three months. Our present thinking is that any flavor compound showing no effects at the 1% level may be considered safe for use in foods. A compound showing an effect at 0.25% is considered suspicious, and one producing toxicity at 0.1% would be classed as too toxic for use. None of the flavors tested so far has shown toxicity of sufficient degree to warrant advising against their use in foods.

As final statement for this part of the discussion it can be said that no intentional food additive presently used in the United States is carcinogenic by the route normally used. Although the pharmacological evidence is not always as complete as it should be to meet all food-safety uncertainties, we see no reason for concern with present usage.

Food Packaging

The contamination of foods with harmful substances whether by direct addition or by indirect addition during processing or from contact with the packaging material is of concern to the Food and Drug Administration. The functions of a package are many and varied. Protection of the food to the point of purchase, protection after the product

reaches the ultimate consumer; prevention of dehydration; retention of flavors and aromas; grease proofing; provision for wet-strength of paper wraps; retardation of rancidity are but a few such functions. To achieve these desired objectives requires the use of synthetic resins, plasticizers, stabilizers, dryers, special coatings, antoxidants, to mention a few. The principal problem involved is whether or not any component of the finished wraps is transferred from the wrapper to the food in contact with the packaging material. As a rule resins are so insoluble as to pose no proble The other ingredients are usually leachable to varying degrees and must be determined. Extractability tests can be conducted using the foods to be packaged, or simpler food type solvents can be substituted. At present approximately 17 resins, 11 plasticizers, 22 stabilizers, 6 release agents, 3 antioxidants, 5 pigments, 4 antimycotics, and 8 lubricants have been established as acceptable for use in food wraps, either by extractability studies, or adequate pharmacological investigations to prove safety, or by both.

General Consideration of Carcinogens in Foods

The introduction of a carcinogen into foods has been the subject of much discussion. If a carcinogen is defined as a substance which will produce a neoplasm in experimental animals regardless of the degree of response, the route of administration, the dosage required, or the time involved, then some chemicals presently used in foods would conform with this definition. We do not believe that a carcinogen is properly so defined. If a chemical is to be administered only by the oral route, the fact that a tumor may be produced by subcutaneous injection would

not rule out its use where oral ingestion only is the mode of entry into the body. In our evaluation of the chronic toxicity, the number of tumors encountered as well as the time and amount of material needed to cause them are considered carefully. Only when the results indicate a significant increase of tumors in animals fed test diets over those fed the control diet is carcinogenicity suspected. Even under these circumstances it cannot be assumed that a chemical which induced tumors in one species of animal by one route will also produce tumors by other routes in this same species or will necessarily be tumorigenic for other animal species. Accordingly, positive results can be taken as creating a suspicion that the chemical under study may be carcinogenic for man, but do not prove it to be so.

MEMORANDUM ON A MEANS OF APPRAISING THE POTENTIAL CANCEROGENIC HAZARD OF THE USE OF PETROLEUM WAXES UNDER CONDITIONS THAT MAY RESULT IN THEIR INTRODUCTION INTO HUMAN FOOD AND BEVERAGES

The question of the safety of the use of refined waxes on food containers was apparently raised in Rome this past summer. According to Dr. Arnold Lehman of the F.D.A., the potential problem was not discussed by Dr. Hueper in his formal paper. (It has since been referred to briefly, among many other sources of contamination of food, in an article in the Saturday Review of literature.) At the same time, the reports in the popular press are sufficient evidence that the subject was discussed informally by some members of the conference. In any event, it seems highly probable that the question will have to be answered eventually by the acquisition and publication of convincing experimental evidence.

On October 4, the following persons participated in an informal discussion of a suitable approach to the problem: Drs. Lehman, Nelson and Voss of the Food and Drugs Administration and Drs. Kehoe and Horton of the Kettering Laboratory. There was unanimous agreement that the somewhat conventional experiment of feeding test materials to rats over a period of two years is not particularly applicable. It was further agreed that the experimental approach to the testing of materials in terms of the responses of animals, must be or, at least, should be controlled in a positive answer by some experimental procedure in which a known carcinogenic hydrocarbon will produce malignant tumors of relevant type and location. In keeping with the sense of the foregoing discussion, and following careful consideration of the entire problem, we propose to investigate the carcinogenic potentialities of

selected materials by introducing them into the forestomach and small intestine of mice (see publications of Lorenz and Stewart of National Cancer Institute) after choosing a suitable solvent for the appropriate experimental material or fraction thereof. The use of positive controls, exposed in corresponding manner to methylcholanthrene in the same solvent, and negative controls, comparably exposed to the solvent alone, would form a necessary part of such a program. (Dr. Lehman and his associates indicated that they would be in substantial agreement with this approach. Dr. Lehman suggested further that, since the methods for developing cancer of the skin from liquid solutions had been worked out, it might be expeditious to apply some of the solutions to the skin, at least in the preliminary phases of the program. The data from the skin, in combination with those from the gastro-enteric tract, would provide a much higher level of confidence in the interpretation of the results then would be possible if only one or the other of the tissues was involved.)

In considering the proper scope of such an investigation, the opinion was general that there is a lack of the data necessary to present a clear picture of the situation rather than an actual or imminent hazard to the public health. Hence, although a fairly detailed examination of the problem seems warranted, it should be carried out in a carefully considered manner and at a logical speed, rather than after the manner of an emergency.

In general, the problem arises out of the fact that refined waxes, as marketed, are faintly to strongly fluorescent under ultraviolet light. While not all fluorescent hydrocarbons are carcinogenic, the converse apparently is true. There is, for example, evidence that the carcinogens responsible for the development of scrotal cancer in wax pressmen are fluorescent.

Since the refined waxes derive from the distillates associated with these occupational skin cancers, the obvious question is raised, since the waxes still fluoresce, whether the carcinogens have been eliminated or to what extent their concentration has been reduced. A program aimed at a qualitative understanding of the composition of the carcinogens in the wax distillates and a quantitative measure of the desired decrease in their concentration during the refining processes seems to be called for.

Although API Research Project MC-1 has been directed primarily at cracked oils, the general principles and techniques developing in that work should be applicable to the wax problem. Biological testing of solutions of suitable fractions of the intermediates and final products in parallel with chemical research on the composition of the fluorescent components should provide the information needed for the long term answer to this problem.

This question concerns every oil company engaged in the production or distribution of petroleum waxes. Since the inclusion of even selected materials from each of these companies would quickly multiply the experimental effort beyond the scope warranted by the nature of the problem, it would seem appropriate to follow the mechanism for sampling used in Project MC-1 in

1949-50. The general boundaries of the investigation would be described by the advisory committee. Guided by this outline, the Kettering Laboratory should assume the responsibility for assembling the necessary confidential information from the individual producers and selecting a representative group of samples. The experience along this line and contacts already established should make this a fairly efficient process of arriving at the desired goal.

As to the general level of the overall effort, an annual expenditure in the neighborhood of $35,000 to $40,000 for about five years would produce the information needed in the most economical and satisfactory manner. It is doubtful in our mind that a higher rate of expenditure initially would be wise or necessary. It will probably be desirable to be able to indicate an early date for the initiation of the investigation. The staff of the Laboratory would be prepared to start the necessary groundwork for the sampling program at a moderate cost, as soon as the decision to proceed has been arrived at.

Robert A. Kehoe

Robert A. Kehoe, M. D.

October 11, 1956

A PROGRAM FOR THE INVESTIGATION OF INTERMEDIATE AND REFINED WAXES

OBJECTIVES: Certain crude paraffinic distillates have been associated with the development of scrotal cancer in wax pressmen. By further refining (essentially deoiling and decolorization processes) the waxy components of these distillates are purified until they meet the specifications for their particular use.

The major objective of the proposed investigation is the determination by suitable biological tests of the stage in the refining process at which the concentration of the components of the paraffinic distillates responsible for their carcinogenic potency for the human skin has been reduced to a negligible level. A correlated and equally important goal will be the development of biological techniques which will yield some estimate of the relative cancer hazard incurred by human beings through the ingestion of these partially or fully refined waxes. Pertinent information on composition obtained in the course of the research on cracked oils and straight run distillates (API Research Project MC-1) will be applicable to the various waxes. Analytical techniques based on these findings will serve as a further check on the extent of the reduction in the concentration of carcinogens present in the crude distillates from which the waxes were derived.

PROCEDURE:

I. Selection of materials

A. Process variables to be considered (partial assembly of this information from individual producers has been carried out in an early phase of Project MC-1)

1. Crude source
2. Preparation of feed to the wax plant
3. Method of deoiling the wax

B. Types of materials under consideration

1. Correlated series of samples
   a. Feed to the wax plant (straight run or extracted paraffinic stocks)
   b. Intermediate scale waxes resulting from sweating or solvent deoiling operations
   c. Refined waxes after final deoiling and decolorization (e.g., MEK-benzol or adsorbents)
2. Waxes of increasing molecular weight (melting point), including those referred to as "microcrystalline"
3. Suitable composites of closely related materials from different producers

C. Pertinent properties by which selected materials may be described

1. Oil content
2. Melting range or molecular weight
3. Content of fluorescent materials (chromatographic)
4. Absorption and mass spectra

II. Biological testing

A. Criteria for selection of experimental conditions

1. Control animals subjected to the administration of vehicles alone, with no added carcinogens, should have a low, essentially negligible incidence of the cancer to be reproduced
2. Positive results should be obtainable if a known carcinogenic hydrocarbon is introduced into the animal under the conditions of the investigation

B. Materials to be tested

1. Solutions of fluorescent fractions (chromatographic) of the waxes, in selected concentrations in the appropriate vehicles
2. Solutions of benzopyrene (BP) or methylcholanthrene (MC) in the same vehicle
3. Vehicles alone
4. Solid solutions of BP or MC in the non-fluorescent fraction (chromatographic) of selected waxes

C. Species of animals to be used

1. Inbred mice for induction of skin cancer
2. Selected species, including mice, which are available from healthy stocks, for induction of cancer of small intestine or other internal organs

II. Biological testing (continued)

D. Experiments involving the induction of skin cancer

1. Tests to determine the relative potencies of liquid fractions of slack waxes which differ with respect to the variables shown in I.A. or I.C.
2. Subsequent tests to determine the relative potencies of liquid fractions, or solutions of fluorescent fractions of related samples of slack wax, intermediate scale waxes, and fully refined wax

E. Experiments involving the induction of intestinal tumors

1. Attempted duplication of published method of induction of intestinal tumors by solutions of MC in olive oil
2. Subsequent attempts to induce similar tumors by solutions of fluorescent fractions of waxes
3. Determination of relative potency to intestine of solid solutions of MC in non-fluorescent fractions of waxes

III. Application of analytical techniques from Project MC-1 to paraffinic oils and separated waxes

Kettering Laboratory
November 1, 1956

December 1, 1956

To: Members of the Medical Advisory Committee, API

From: Dr. John J. Phair

The accompanying statement describes the present status of the epidemiological studies conducted for the Medical Advisory Committee. This part of the report could not be prepared in time for distribution prior to the November meeting of the Medical Advisory Committee, but it should be attached to and considered as a section of the document circulated by Dr. Horton summarizing the biological and chemical studies.

John J. Phair, M. D.

Attachment